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PGC1α Agonist Rescues Doxorubicin Induced Cardiomyopathy by Mitigating the Oxidative Stress and Necroptosis

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Abstract: Cardiomyopathy (particularly dilated cardiomyopathy (DCM)) significantly contributes to development and progression of heart failure (HF) and inflammatory factors further deteriorate the symptoms. Morphological and functional defects of heart in doxorubicin (DOX) induced cardiomyopathy (cardiotoxicity) are similar to those of DCM. We used PGC-1α (PPAR-γ coactivator-1α) agonist that is considered as the 'master regulator' of mitochondrial biogenesis with an aim to rescue the DOX induced deleterious effects on heart. Forty male C57BL/6J mice (8 weeks old) were divided in four groups, Control, DOX, ZLN005 and ZLN005 + DOX (n = 10 each group). The DOX induced (10 mg/kg single dose) cardiomyopathy mimics DCM like phenotype with marked morphologic alteration in cardiac tissue and functional derangements. Significant increased staining observed for Masson Trichome/Picrosirius red and α-SMA that indicated enhanced fibrosis in DOX group compared to control that was attenuated by PGC-1α agonist (four doses of 2.5mg/kg/dose; cumulative dose=10mg/kg). Similarly, elevated expression of necroptosis markers along with enhanced oxidative stress in DOX group were alleviated by PGC-1α agonist. These data collectively suggested the potent therapeutic efficacy of PGC-1α agonist in mitigating the deleterious effects of DOX induced cardiomyopathy and it may be targeted in developing the future therapeutics for the management of DCM/HF.

Keywords: Cardiomyopathy; DCM; PGC-1α; oxidative stress; necroptosis; fibrosis

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1. Introduction

Majority of the cardiomyopathy cases advances into heart failure, where terminal treatment is heart transplantation, which is challenging due to limited availability of donors. Among cardiomyopathy the largest prevalence is of DCM phenotype, which accounts for more than 50% of heart transplantation cases [1] and is characterized by increased ventricular dimensions, thinning of chamber walls, reduction in ejection fraction and overall compromised systolic function. DCM can be genetic, acquired, drug induced or idiopathic [2].

Owing to significant cardiotoxicity properties, use of chemotherapeutic drug like DOX is a major concern among cancer patients. The DOX treated patients often presented with many life threatening cardiovascular complications, prominent among which is cardiomyopathy (or DCM) [3]. Clinically, the DOX induced cardiomyopathy is having similar morphological and functional derangements in the cardiac tissue to those of DCM that eventually culminating to HF. However, despite extensive research, the precise mechanism of DOX induced cardiomyopathy (or DCM) is not yet completely understood. Cell death is the most unifying event that occurs in majority of heart injuries

associated with disease conditions like ischemia-reperfusion, cardiomyopathy, myocardial infarction, heart failure including DOX. The three well known causative factors of cell death are, (i) excessive oxidative stress due to massive production of reactive oxygen species (ROS); (ii) interaction of DOX with topoisomerase- α and -β by DNA intercalation thereby causing double strand breaks and hindering transcription machinery; (iii) mitochondrial damage [4-8].

Besides apoptosis, there are various cell death mechanisms that have been explained as regulated cell death pathways e.g. autophagy, necroptosis, pyroptosis and ferroptosis [9, 10]. In recent studies, above mentioned cell death pathways have been widely associated with DOX induced cardiomyopathy [11] but which cell death pathway predominantly triggered by DOX and the underlying mechanism is not clearly defined. Recently, necroptosis has gained wide attention and its role has been implicated in DOX induced cardiomyopathy/cardiotoxicity [11-13]. Necroptosis has been identified as cell death mechanism that occurred in a programmed way, and it is regulated by kinases i.e. receptor interacting protein kinases (RIPK)-1, RIPK-3, and (mixed-lineage kinase and domain-like pseudokinase (MLKL).

Further, necroptotic cells releases 'alarmin' molecules which act as danger signals for nearby cells [14, 15]. Some of these molecules are ubiquitously present and have important role in cellular functions, but ones outside cells, these act as danger signal for neighboring cells [14]. Alarmins are potent source of inflammation and augments the recruitment of inflammatory immune cells and can also trigger cell death. Enhanced inflammatory milieu at the site of pathology augments oxidative stress leading to exacerbated tissue damage.

Therefore, strategies to annihilate the DOX induced cardiomyopathy via regulating the necroptosis process, alarmin release and reducing oxidative stress are highly warranted and the outcome of such studies may be applied in the management of different types of cardiomyopathies/cardiac injuries to prevent its progression towards HF. Targeting necroptosis resulted in protective effects on doxorubicin-induced cardiotoxicity models [9, 10]. Considering the above facts, in this study we targeted a nuclear encoded transcriptional coactivator i.e. PGC-1α (peroxisome proliferator-activated receptor gamma ((PPAR-γ) coactivator-1alpha) which is considered as the 'master regulator' of mitochondrial biogenesis and its function. Further, PGC-1α exerts a strong antioxidant effect [16], regulates cellular energy metabolism, cell growth [17] and its role has also been suggested in driving inflammation and immune regulation [18]. Activation of PGC1α/SIRT (Sirtuin)1 pathway has been reported to be associated with elevation of autophagy/mitophagy leading to suppression of oxidative stress-mediated ROS production [19]. However, the role of PGC-1α in regulating necroptosis, alarmin production and oxidative stress in doxorubicin induced cardiomyopathy model has not been described. Therefore, present study employed PGC-1α agonist (i.e. ZLN005) with an aim to rescue the DOX induced deleterious effects on heart via alleviating the necroptosis and oxidative stress thereby restoring the cardiac function by preventing extensive cardiac tissue remodeling.

2. Materials and Methods:

2.1. Mice and Experimental Procedures

All animal experiments were approved by Institute Animal Ethics Committee, All India Institute of Medical Sciences (AIIMS), New Delhi. C57BL/6 male mouse of 24-27g (8 weeks old) were purchased from Central Animal Facility, AIIMS, Delhi. Mouse were fed ad libitum and were housed under standard 12 h light-dark cycles with controlled temperature and humidity in ventilated rooms. Daily performance of mice, their weight and behavior were recorded. At the end of treatment regime mice were euthanized humanely with overdose of isoflurane. The hearts were perfused, isolated and used for subsequent experiments. Forty mice were randomly divided into four groups i.e. con-

trol, DOX, ZLN005 and ZLN005+DOX (n = 10, each group). The doxorubicin model was established as previously described [20]. Briefly, doxorubicin (single dose, 10mg/kg, i.p., Cayman Chemicals, USA) was administered (**Figure 1a**) in DOX group and survival schedule of 7 days was followed. Control mice were administered with vehicle (1X PBS, i.p.). The ZLN005 + DOX group received DOX and ZLN005 whereas ZLN005 group received ZLN005 only. Repeated dosing model was adapted for ZLN005 based intervention [21]. The ZLN005 (four doses of 2.5mg/kg/dose, cumulative dose, 10mg/kg, Cayman Chemicals, USA) was administered i.v., 2hrs after doxorubicin [22] through retro-orbital route every alternate day as demonstrated in **Figure 3a**.

2.2. Gene expression by quantitative PCR

Heart tissues were homogenized using mortar and pestle in liquid nitrogen and transferred to TriExtract (G-Biosciences). Total RNA was isolated and quantified followed by DNase treatment (DNase I, RNase-free, ThermoFisher Scientific, CA, U.S.A.). Complementary DNA (cDNA) was synthesized using cDNA synthesis kit (iScript cDNA synthesis kit, BioRad, Hercules, CA, USA) following manufacturer's instructions. The PCR was performed using iTaq Universal SYBR Green Supermix (Biorad, Hercules, CA, USA) in CFX96 Real-time PCR system, (BioRad, Hercules, CA, USA) in accordance to MIQE guidelines. PCR cycles were set for 10 min denaturation, followed by 40 cycles of denaturation at 95 ℃ for 15secs, followed by primer annealing and extension at optimized temperature. Samples were run in triplicates and GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) was used as housekeeping gene for normalization of data. The gene expression data was represented as $2^{-\Delta Ct}$ for all the groups.

Table 1. List of genes and the primers.

S.No.	Gene name	Accession ID	Forward Primer (5′→ 3′) Reverse Primer (5′→ 3′)
1.	*CATHEPSIN-B*	NM_007798.3	GGCTCTTGTTGGGCATTTGG
			CAGCTTCACAGCTCTTGTTGC
2.	*HMGB1*	NM_001313894.1	TCCCTCATCCTTGTTTACTCG
			GCAGTTTCCTATCGCTTTGG
3.	*RIPK1*	NM_001359997.1	AGGTGTCCTTGTGTTACC
			CCTCCACGATTATCCTTCC
4.	*RIPK3*	NM_019955.2	AAGACAGTCCTTGCCACTTCC
			TGGGTCAAGAGTCAGTTTGGG
5.	*MLKL*	NM_001310613.1	ATGCCAGCGTCTAGGAAACC
			TCGGGCAGGTTCTTCTTTCC
6.	*S100B*	NM_009115.3	ACAACGAGCTCTCTCACTTCC
			CATCTTCGTCCAGCGTCTCC
7.	*PGC-1α*	NM_008904.3	GCACACACCGCAATTCTCC
			AGGCTTCATAGCTGTCGTACC
8.	*GAPDH*	NM_001411843.1	AACTTTGGCATTGTGGAAGGG
			CATCACGCCACAGCTTTCC

2.3. Immunofluorescence

Freshly isolated mouse hearts were dissected on ice and immediately fixed in 4% paraformaldehyde. Tissue was paraffin embedded and sectioned at 3-5 microns. The sections were deparaffinized followed by antigen retrieval and blocking. Primary antibody incubation with RIPK1 (H-207) (1:500, Santa Cruz), RIPK3 (H-43) (1:500, Santa Cruz), MLKL (1:500, Thermo Fisher Scientific), alpha-SMA (1: 500, Abcam), PGC-1α (1:200, ABclonal) at 4°C was done overnight, followed by washing and secondary antibody incubation with FITC-conjugated goat anti-rabbit (1:500, Abcam) for one hour at room temperature, followed by nuclei counterstain with 4',6-diamidino-2-pheylindole (DAPI)

(Sigma Aldrich). Tissue sections were washed and mounted using Vectashield antidfade mounting medium (Vector Laboratories, Newark, USA). Images were captured using Nikon Eclipse Ni upright fluorescent microscope (Nikon, Japan) using NIS-Elements Br software (Nikon, Japan). Mean fluorescence intensity of signal and background was calculated using Fiji software (NIH, USA). The data from 4 different sections was used to capture five different fields of views each, which were used for further statistical analysis.

2.4. Histological analysis

For Trichome staining sections were deparaffined in decreasing gradient of ethanol, fixed in Bouin's solution (Sisco Research Laboratories Pvt. Ltd., India) overnight. Sections washed in running tap water followed by staining of nucleus using modified Weigert's Iron hematoxylin stain (Sisco Research Laboratories Pvt. Ltd., India) which was washed and staining was proceeded for trichome stain to discriminate between muscle and fibrosis, followed by dehydration and mounting. Similarly, for Picrosirius Red staining the deparaffinized tissue sections were stained using modified Weigert's hematoxylin (Sisco Research Laboratories Pvt. Ltd., India) which was washed to stain for Sirius Red (Sisco Research Laboratories Pvt. Ltd., India) for one hour, which was proceeded by dehydration and mounting of tissue using DPX Mountant (Sigma, USA).

2.5. Protein quantification by Enzyme Linked Immune Sorbent Assay (ELISA)

Protein quantification was performed using ELISA. Mouse 8-hydroxydesoxygaunosine (8-OHdG) (MyBioSource, USA), Malondialdehyde (Elabscience), Catalase (MyBioSource, USA), Superoxide Dismutase (MyBioSource, USA), Glutathione (GSH, yBioSource), Glutathione Reductase (GSSG, (MyBioSource, USA), Collagen-I (MyBioSource, USA,) Collagen III (MyBioSource, USA), Fibronectin (MyBioSource, USA), Laminin (MyBioSource, USA) kits were used and all the immunoassay steps were performed as per manufactures instructions. Optical density was measured using Epoch microplate reader (BioTek, Agilent) at 450nm. The concentrations were expressed as picogram (pg) or nanogram (ng) per milliliter (mL).

2.6. Statistical analysis

All data was analyzed using graph Pad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA). Data was analyzed for normality using D'Agostino and Pearson test, Shapiro-Wilk test, Kolmogorov-Smirvov test and visually validated by plotting Q-Q plot. Mann Whitney U test was done for non-parametric tests (qPCR data). One-way ANOVA (ELISA data) and unpaired t test (Immunofluorescence mages quantification) was performed for normally distributed data. Significance test was performed using p-value of <0.05, error bars indicated mean ± SD (standard deviation). All experiments were performed in triplicates and the average was used for subsequent analysis.

3. Results

Doxorubicin is a widely used chemotherapeutic drug. It demonstrates dose dependent toxicity. We have used an acute cardiotoxicity model with short term survival (**Figure 1a**). Overall the heart showed enhanced dilatation of chambers and higher filling capacity as quantified by histology images (**Figure 1b**). Alterations in cardiac tissue morphology showed enhanced vacuolations, distorted myofibril structure and increased leukocyte infiltration (**Figure 1c**).

We analyzed fibrosis in cardiac tissue by Masson Trichome, Sirius Red and α-SMA (alpha-smooth muscle actin) staining and significantly increased staining revealed enhanced fibrosis by all the three methods in DOX group compared to controls (**Figure 2a**). Further, necroptosis cell death pathway was analyzed by immunofluorescence based protein localization (and quantification) for key molecules like RIPK1, RIPK3 and MLKL

in cardiac tissue. Significantly increased staining (p<0.0001) was observed for all three markers in DOX group compared to controls that was indicative of enhanced necroptosis (Fig 2c and 2d). However, corresponding mRNA levels showed differential expression pattern that were not statistically significant (**Figure 2c**).

We identified PGC1α agonist i.e. ZLN005 that significantly increased the expression of PGC1α protein (no significant changes observed at mRNA levels) in the heart tissue **(Figure S1, supplementary data)**. In order to rescue the DOX induced cardiomyopathy phenotype, we treated mice with ZLN005 as mentioned in above section (**Figure 3a**). We observed remarkable recovery in myocardial mass, reduced dilatation of heart and vacuolations with overall improvement in structural integrity in ZLN005+DOX group compared to DOX group (**Figure 3b and 3c**). Weight reduction, a critical parameter to determine the overall health and metabolism of mice also revealed significant improvement in ZLN005+DOX group when compared to doxorubicin treated group (**Figure 3d**). Further, marked decrease in Masson's Trichome, Sirius Red and α-SMA staining (**Figure 4a**) were observed in ZLN005+DOX treated group compared to DOX group. Significant reduction (p<0.0001) in immunofluorescence based protein localization of necroptosis markers was observed in ZLN005+DOX treated group compared to DOX group indicating enhanced survival of cardiomyocytes attributed by PGC-1α agonist (**Figure 4c and 4d**). However, no significant difference was observed in the corresponding transcript levels when compared all three groups **(Figure 4b)**. Next, the transcript expression levels of alarmin molecules i.e. HMGB1, S100b and Cathepsin-B were determined but statistically significant difference was not observed for all three studied genes in all four groups (**Figure 5**).

Since DOX triggered a potent oxidative stress response, we further investigated the status of hallmark markers associated with oxidative stress response to understand the ZLN005 effect. We analysed the levels of 8-OHdG (a marker of DNA damage) (**Figure 6a and 6b**) and MDA (a lipid peroxidation marker) that were found to be significantly increased in DOX group compared to controls (p<0.0001) and corresponding levels were significantly reduced in ZLN005+DOX (p=0.0004 for 8-OHdG and p=0.016 for MDA) but remained higher compared to control group. Conversely, the ROS (reactive oxygen species) scavenging enzyme i.e. SOD and catalase activities were significantly decreased in DOX group compared to control (p<0.0001) and activities of same were increased after ZLN005 treatment (DOX+ZLN005 group) but they were comparable to control group (**Figure 6c and 6d**). Similarly, glutathione (GSH) levels and Glutathione Reductase (GSSG) were respectively decreased (p=0.003) and increased (p=0.004) in DOX group compared to control, while contrasting trends were observed in DOX+ZLN005 group (**Figure 6e and 6f)** compared to DOX group. Likewise, the GSH:GSSG ratio was decreased DOX group compared to control (p=0.001) and it was restored to normal in DOX+ZLN005 group (p=0.001) (**Figure 6g**).

Based on high α-SMA and fibrosis staining that indicated significant tissue remodeling, we further investigated the effect on cardiac tissue remodeling by quantifying the levels of key extracellular matrix proteins involved in structural homeostasis i.e. Laminin, fibronectin, collagen I and collagen III. We observed significant upregulation of laminin and fibronectin in DOX group compared to control group (p<0.0001) but their levels remained comparable to control in ZLN005+DOX group, (**Figure 6h and 6i**). Next, as expected the collagen-I and collagen III were significantly decreased and increased respectively in DOX group (p<0.0001) compared to controls, but these alterations were preserved by ZLN005 treatment as evident by ratio of collagen I to collagen III (**Figure 6l**). In all the above studied parameters, the control groups were found comparable to ZLN005 group.

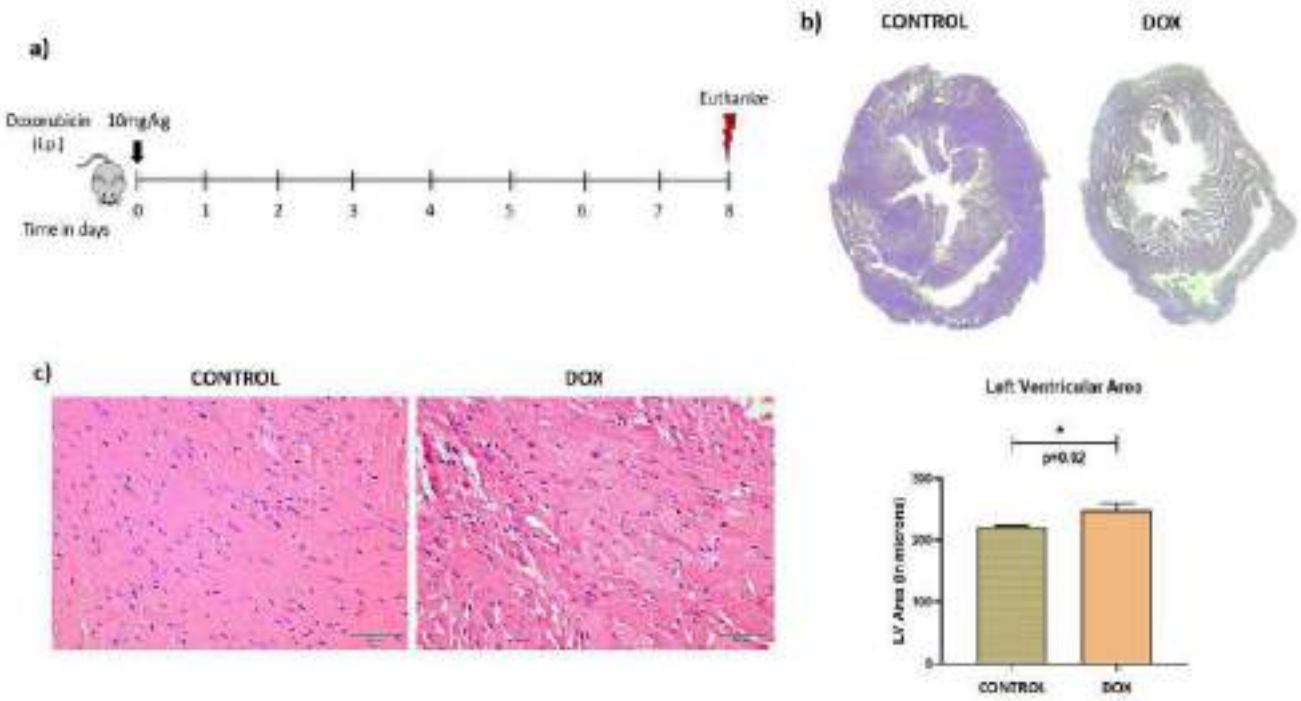

Figure 1. Doxorubicin induced model of cardiomyopathy. (**a**) Schematic diagram of dose schedule for doxorubicin induced cardiomyopathy model. (**b**) Micrograph of cardiac tissue (upper panel) and quantification of Left ventricular Volume (lower panel). (**c**) Hematoxylin and eosin staining of control and doxorubicin treated heart. Scale bar = 50μm, (*) = $p < 0.05$.

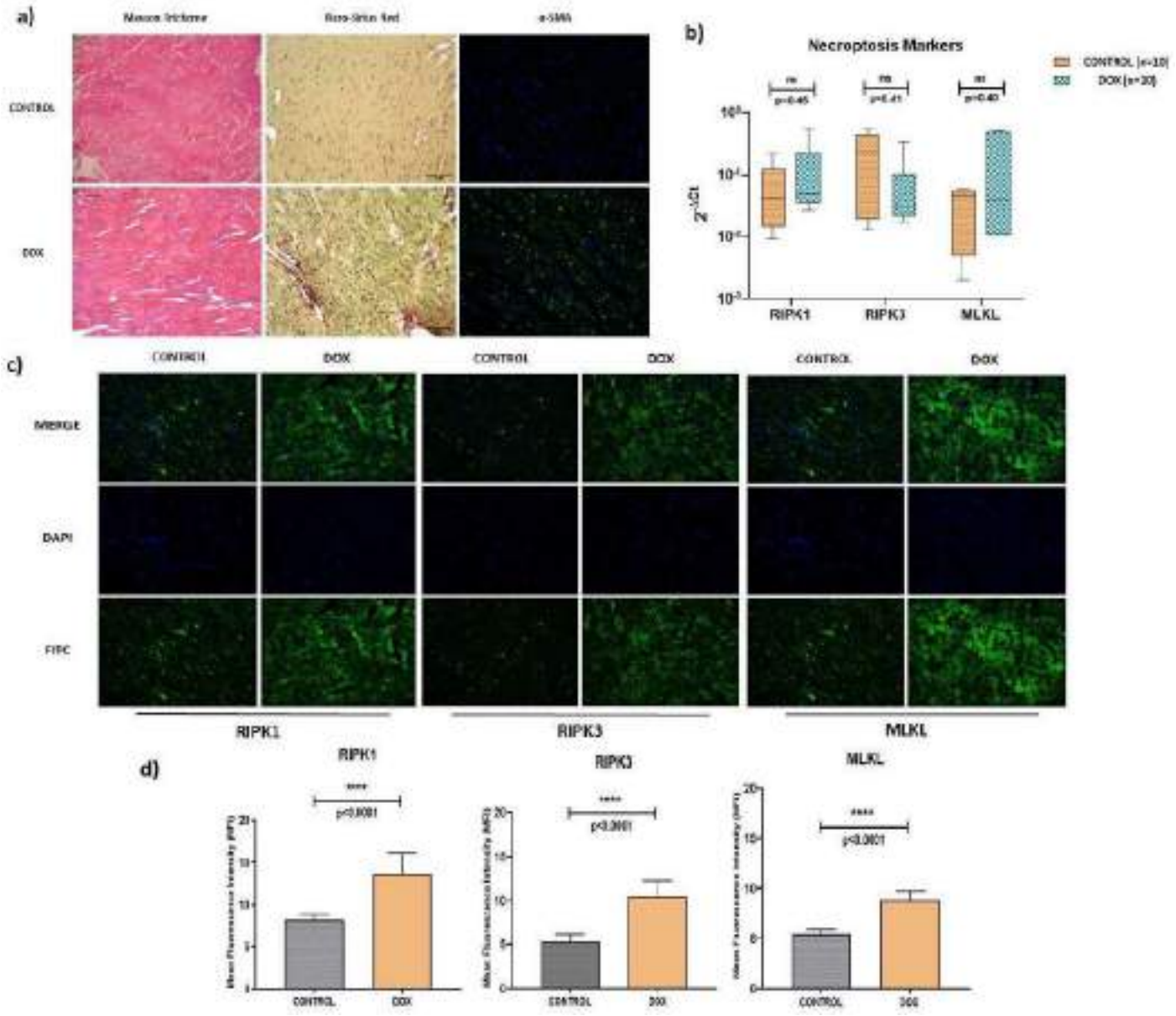

Figure 2. (**a**) Analysis of cardiac tissue fibrosis via Masson Trichome Staining (Muscle tissue = pink to red, Collagen deposition = Blue and Nucleus = Black), Pirco-Sirius Red staining (Muscle tissue and cell cytoplasm = yellow, collagen deposits = Red and nucleus = black) and A-SMA (blue = nucleus and green = α-SMA). (**b**) qPCR based transcripts expression for necroptosis markers (RIPK1, RIPK3, MLKL). (**c**) Immunofluorescence of Necroptosis markers (RIPK1, RIPK3 and MLKL) in control and doxorubicin treated groups. (**d**) Quantification of immunofluorescence data. ns= non-significant, (*) = $p<0.05$, (**) = $p<0.01$,(***) =$p<0.001$, (****)= $p<0.0001$.

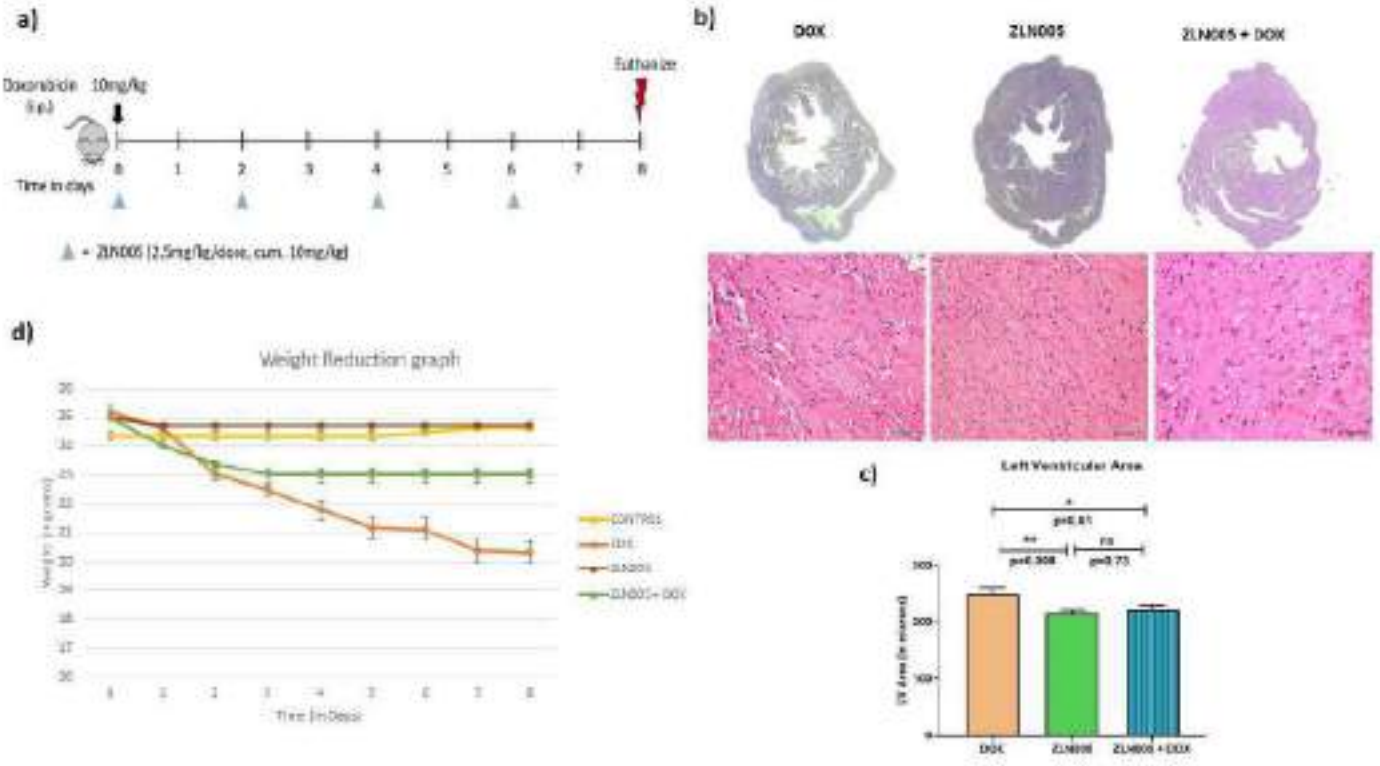

Figure 3. PGC-1α agonist (ZLN005) based intervention to rescue doxorubicin induced model of cardiomyopathy. **a**) Schematic diagram of dose schedule for ZLN005 based intervention in doxorubicin induced cardiomyopathy model. **b**) Micrograph of cardiac tissue (top) and haematoxylin and eosin staining (bottom) of doxorubicin treated heart and intervention group. Scale bar = 50μm. **c**) Quantification of Left ventricular Volume. **d**) Comparison of body weights of mice with time (in days) in all the study groups, (*) = $p < 0.05$.

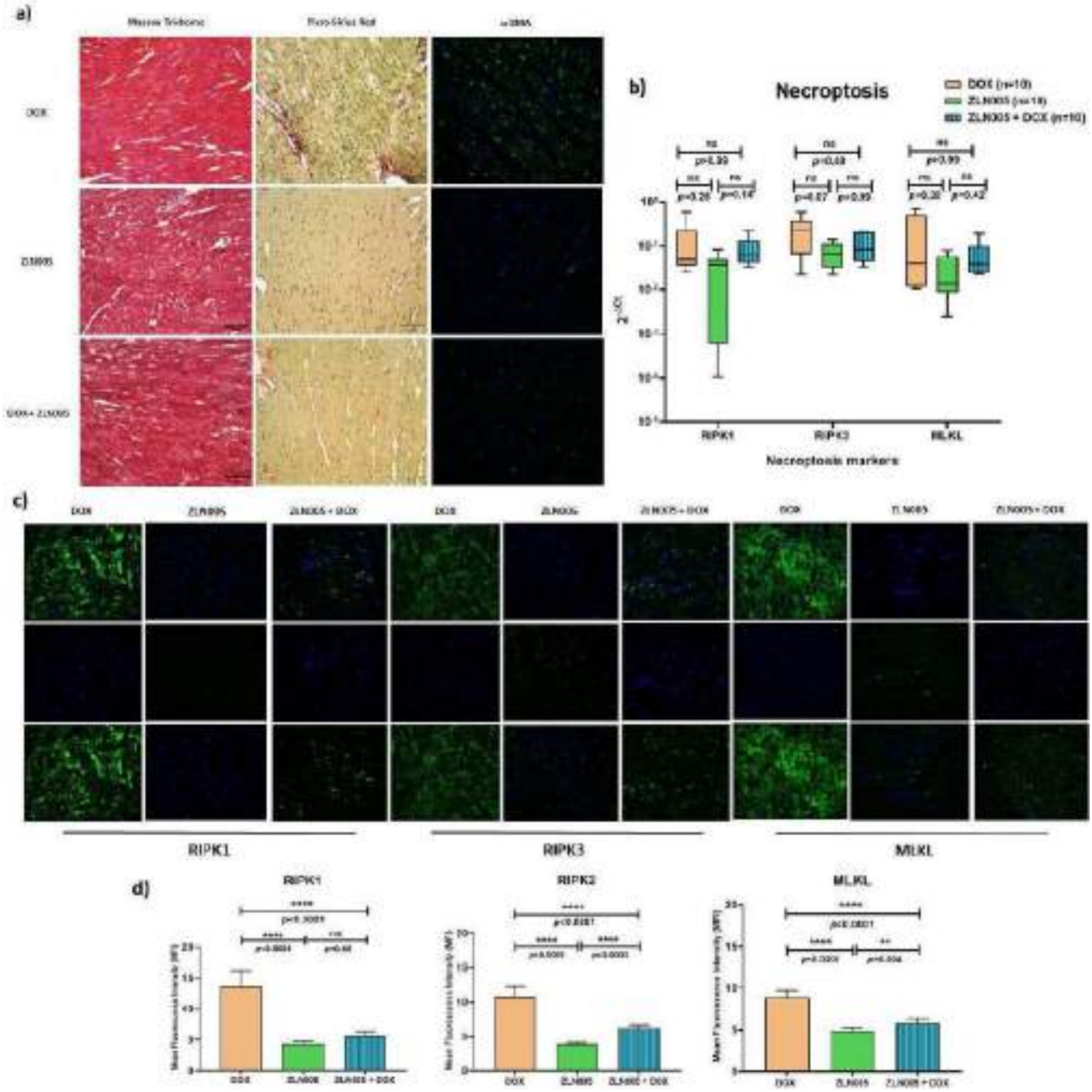

Figure 4. a) Analysis of fibrosis via Masson Trichome Staining (Muscle = pink to red, Collagen = Blue and Nucleus = black), Pirco-Sirius Red staining (Muscle tissue and cell cytoplasm = yellow, collagen = red strands and nucleus = black) and α-SMA(blue = nucleus and green = staining of α-SMA) in Dox, ZLN005 and ZLN005+Dox groups. **b**) qPCR based transcripts expression for necroptosis markers (RIPK1, RIPK3, MLKL). **c**) Immunofluorescence of Necroptosis markers (RIPK1, RIPK3 and MLKL) in study groups. **d**) Quantification of immunofluorescence data. ns= non-significant, (*) = p<0.05, (**) = p<0.01,(***) = p<0.001, (****)=p<0.0001.

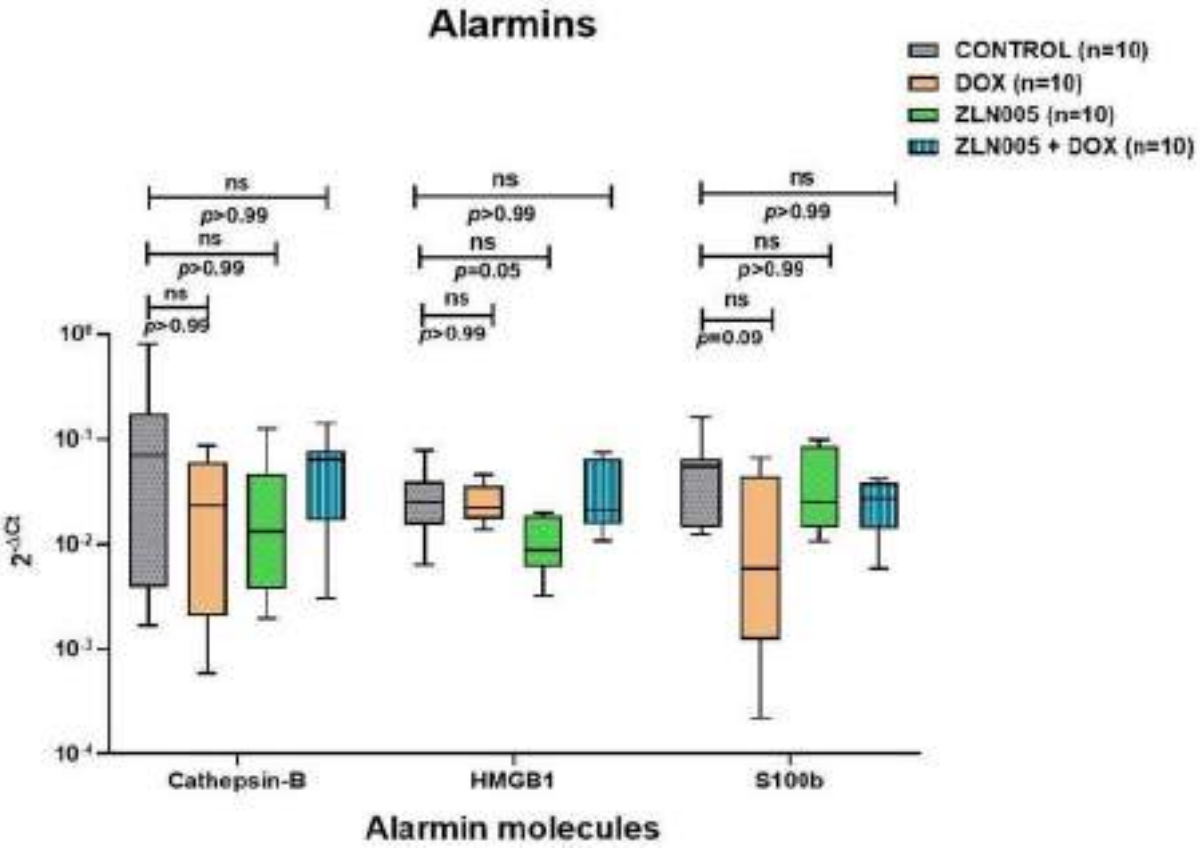

Figure 5. Transcript expression levels (qPCR) of alarmin molecules in control, Dox, ZLN005 and ZLN005+Dox hearts. ns= non-significant (significant p value is set as p <0.05).

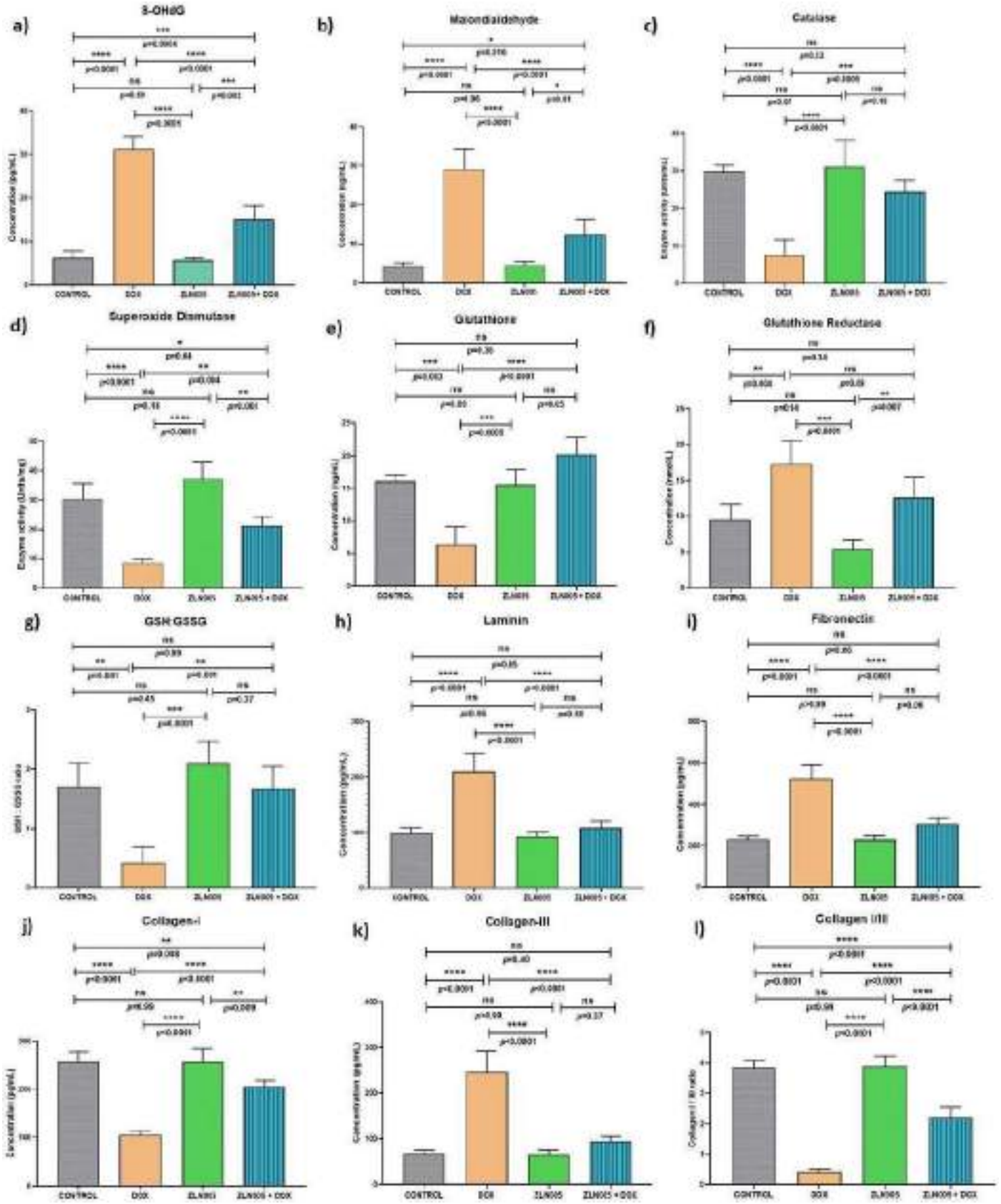

Figure 6. a-g) Status of Oxidative stress markers in cell lysates of cardiac tissue of control, Dox, ZLN005 and ZLN005+Dox groups. **h- l)** Tissue remodeling parameters for cardiac cell lysates of all the study groups. 8-OHdG = (8-oxo-7,8,-dihydro-2′-deoxyguanosine). (*)= $p<0.05$, (**)=$p<0.01$, (***)=$p<0.001$, (****)=$p<0.0001$.

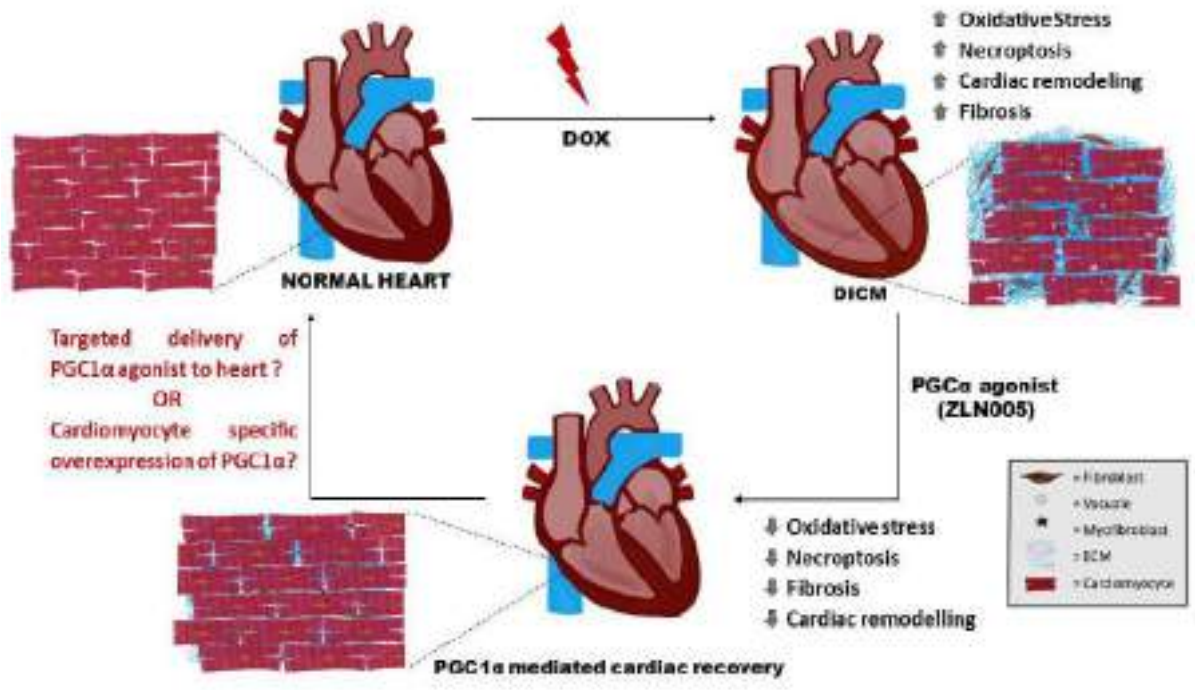

Figure 7. Schematic representation of DOX induced cardiotoxic effects (enhanced oxidative stress, necroptosis, fibrosis and cardiac remodeling) which was mitigated by PGC1 α agonist i.e. ZLN005. (? = require further investigation). DOX= doxorubicin, DICM = DOX induced cardiomyopathy.

4. Discussion

Heart failure (HF) remains a global health issue that requires development of newer treatment strategies to combat the alarmingly increasing disease burden. As mentioned above significant proportion of cancer survivors who received chemotherapy (e.g. DOX) were also presented with various types of cardiomyopathies [4-6] and at present dexrazoxane is the only clinically approved agents used to minimize the DOX induced cardiotoxicity [12].

Since DOX causes mitochondrial dysfunction and mitochondrial homeostasis is critical to meet the high energy demand of a dynamic organ like heart [23, 24], it is imperative to target a molecule that will compensate the DOX mediated toxic effect on mitochondrial dynamics along with reducing the oxidative stress and cell death.

As described earlier, PGC1α is a master regulator of mitochondrial biogenesis and it is also essential for the cardiomyocytes during developmental stages. It has been reported as a crucial factor for differentiation/maturation of human embryonic stem cells (hESC) into cardiomyocyte phenotype [25] and it is also known to regulate the respiration process in hiPSC (human induced pluripotent stem cells) generated cardiomyocytes [26]. Role of PGC1α in mitigating cardiac injury is however, not well defined particularly in context with necroptosis, oxidative stress and cardiac tissue remodeling. Decreased PGC1α expression is reported in doxorubicin induced model of cardiotoxicity [8] and in *in vitro* models of cardiac hypertrophy [27]. Further, enhancing PGC1α in renal I/R injury model was able to reverse fibrosis [28] and rescue nephrotoxicity. Similar observations were observed in neurotoxicity I/R model [22]. Liang et al. reported that the oxidative damage and mitochondrial dysfunction induced by H_2O_2 exposure in IPEC-1 intestinal cells has been ameliorated by activation of PGC1α/SIRT1 pathway *via* upregulating autophagy/mitophagy [19] indicating the critical role of PGC1α in regulating oxidative stress and mitochondrial function. Therefore, we employed a PGC1α agonist with an aim to rescue the DOX induced cardiomyopathy like phenotype. Our findings revealed an increased cardiac tissue mass and improved structural changes with reduced vacuo-

lations and fibrosis pointing towards overall improvement in DOX induced injury and the data was found in agreement with above mentioned studies in different disease models. Since, PGC1α possess potent anti-oxidant properties and its role has been demonstrated in regulating the cellular growth and mitochondrial antioxidant defense system in distinct cell types under various stressors influence/disease conditions [16, 19, 29, 30]. In the present study ZLN005 treatment mitigated the harmful effects of DOX induced oxidative stress (kept *status quo* with control group) with corresponding improvement in cardiac fibrosis and preserved structural integrity.

Enhanced activities of free radical scavenging enzymes i.e., Catalase, SOD and GSH and decreased concentration of 8-OHdG (DNA damage marker), MDA (lipid peroxidation marker) and GSSH observed in ZLN005 intervention (ZLN005+DOX) group compared to DOX group and levels of catalase, glutathione, glutathione reductase, GSH:GSSG ratio of ZLN005+DOX group was found comparable to control group.

8-OHdG increases with oxidative stress and ROS accumulation, thereby contributing to DNA damage and is related to cardiovascular disease onset and progression [31, 32]. Status of key antioxidant enzymes including catalase, glutathione and superoxide dismutase were enhanced in ZLN005+DOX group. Catalase overexpression in heart prevents progression into heart failure and influences myocardial remodeling by reducing fibrosis [33]. The SOD serves as first line of defense against ROS and SOD levels related to adverse LV geometry and progression towards HF [34] and similar findings were observed in the DOX model of present study. Increased SOD levels are associated with cardioprotective effect [35], and similar outcomes were observed in ZLN005+DOX group.

Alarmins are a set of ubiquitously present molecules mediating diverse physiological roles. Alarmins may play a dual role that can be protective or detrimental for cells in spatio-temporal context. Cell survival and proliferation is regulated by S100b, it has been shown to promote neuronal cells survival in picomolar to nanomolar quantities and cell death in micromolar concentrations [36]. Further, stimulation with S100b inhibits myogenic differentiation [37] and post DOX treatment; cardiomyocytes have a tendency to differentiate into fibroblast like phenotype with corresponding decrease in S100b levels. Intracellular S100b reportedly inhibited apoptosis in myoblast while playing a role in their differentiation [38]. In our present study, we observed similar trends where *S100b* transcript levels were decreased in DOX group and increased after ZLN005 (ZLN005+DOX group) treatment but the comparison was not statistically significant. HMGB1 is another alarmin investigated and is responsible for mediating cardiac injury [39, 40]. In contrast to our study i.e. DOX (10mg/kg, i.p.) with 1 week survival, Yao et al. administered DOX (20mg/kg, i.p.) with survival of 5 days [40] where they found increased HMGB1 protein expression in cardiac tissue along with increased circulating HMGB1 concentration in serum of DOX group compared to control group. Another study demonstrated differential binding pattern of HMGB1 to DNA with dose dependent response of DOX i.e. increased HMGB1-DNA binding at low DOX concentration and *vice versa* [41]. Cathepsin–B is a lysosomal protease that is associated with aggravation of symptoms in DOX injury model [42], Liu et al. reported increased cathepsin-B protein expression in cardiomyocyte cell line H9C2, post DOX (0.5uM, 24h) treatment [42]. Cathepsin-B is also known to mediate cardiac remodeling events [43] and play a role in progression of cardiomyopathy phenotype through cell death pathways [44]**.** However, in our study, we observed no significant variation in transcript expression levels of *HMGB1* and *cathepsin-B* in cardiac tissue and corresponding protein levels will be required to address these discrepancies.

During extracellular matrix remodeling components like fibronectin and laminin play an important role in deposition of collagen fibers within myocardium. This leads to extracellular matrix protein accumulation leading to stiffening of myocardium. Inhibition of fibronectin improves cardiac dysfunction and halts progression towards heart failure [45]. In our present study we found significant increased concentration of laminin

and fibronectin proteins in heart tissue lysates in DOX group and it was restored to normal levels after ZLN005 treatment thereby circumventing the harmful DOX induced tissue remodeling process. Further, Col I/III ratio is an important factor involved in tissue remodeling associated with myocardial infarction and progression toward HF [46, 47]. In the present study, we found decreased Col I/III ratio in DOX group compared to control. The ZLN005 (ZLN005+DOX group) treatment improved the Col I/III ratio towards the control. All these data collectively demonstrated the potential tissue remodeling effect of PGC1α that has not been reported previously.

The present study has not reported the data related to cardiac function. The echocardiography based cardiac function data (e.g. ejection fraction (EF), LVEDD (left ventricular end-diastolic dimension), LVESD (left ventricle end-systolic dimension), FS (fractional shortening) etc.) would be of great interest to understand the effect of PGC1α agonist on the functional cardiac parameters and it is one of the limitations of the present study.

5. Conclusion

In summary, this study described the role of PGC1α agonist (ZLN005) in mitigating cardiomyopathy phenotype by strengthening the redox balance via mitigating the DOX mediated oxidative stress, preventing the harmful tissue remodeling effects and necroptosis. However, a comprehensive study is required to understand the holistic effect of PGC1α agonist and PGC1α mediated regulatory effects on heart. As described in Figure 7, a targeted delivery of PGC1α agonist in heart or cardiomyocyte specific overexpression of PGC1α may enhance the therapeutic outcome in the present experimental set-up. The outcome may be translated to the clinical set-up for the management of various cardiac injury based disease conditions and/or to prevent their progression towards HF.

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References:

1. Primo, G.; Le Clerc, J. L.; Goldstein, J. P.; De Smet, J. M.; Joris, M. P., Cardiac transplantation for the treatment of endstage ischemic cardiomyopathy. *Advances in cardiology* **1988,** *36*, 293-7.
2. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. **1996,** *93* (5), 841-842.
3. Hudson, M. M.; Ness, K. K.; Gurney, J. G.; Mulrooney, D. A.; Chemaitilly, W.; Krull, K. R.; Green, D. M.; Armstrong, G. T.; Nottage, K. A.; Jones, K. E.; Sklar, C. A.; Srivastava, D. K.; Robison, L. L., Clinical ascertainment of health outcomes among adults treated for childhood cancer. *Jama* **2013,** *309* (22), 2371-2381.
4. Volkova, M.; Russell, R., 3rd, Anthracycline cardiotoxicity: prevalence, pathogenesis and treatment. *Current cardiology reviews* **2011,** *7* (4), 214-20.
5. Swain, S. M.; Whaley, F. S.; Ewer, M. S., Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer* **2003,** *97* (11), 2869-79.
6. Lefrak, E. A.; Piťha, J.; Rosenheim, S.; Gottlieb, J. A., A clinicopathologic analysis of adriamycin cardiotoxicity. **1973,** *32* (2), 302-314.
7. Shi, Y.; Moon, M.; Dawood, S.; McManus, B.; Liu, P. P., Mechanisms and management of doxorubicin cardiotoxicity. *Herz* **2011,** *36* (4), 296-305.
8. Zhang, S.; Liu, X.; Bawa-Khalfe, T.; Lu, L.-S.; Lyu, Y. L.; Liu, L. F.; Yeh, E. T. H., Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nature Medicine* **2012,** *18* (11), 1639-1642.
9. Tang, D.; Kang, R.; Berghe, T. V.; Vandenabeele, P.; Kroemer, G., The molecular machinery of regulated cell death. *Cell Research* **2019,** *29* (5), 347-364.
10. Galluzzi, L.; Vitale, I.; Aaronson, S. A.; Abrams, J. M.; Adam, D.; Agostinis, P.; Alnemri, E. S.; Altucci, L.; Amelio, I.; Andrews, D. W.; Annicchiarico-Petruzzelli, M.; Antonov, A. V.; Arama, E.; Baehrecke, E. H.; Barlev, N. A.; Bazan, N. G.; Bernassola, F.; Bertrand, M. J. M.; Bianchi, K.; Blagosklonny, M. V.; Blomgren, K.; Borner, C.; Boya, P.; Brenner, C.; Campanella, M.; Candi, E.; Carmona-Gutierrez, D.; Cecconi, F.; Chan, F. K. M.; Chandel, N. S.; Cheng, E. H.; Chipuk, J. E.; Cidlowski, J. A.; Ciechanover, A.; Cohen, G. M.; Conrad, M.; Cubillos-Ruiz, J. R.; Czabotar, P. E.; D'Angiolella, V.; Dawson, T. M.; Dawson, V. L.; De Laurenzi, V.; De Maria, R.; Debatin, K.-M.; DeBerardinis, R. J.; Deshmukh, M.; Di Daniele, N.; Di Virgilio, F.; Dixit, V. M.; Dixon, S. J.; Duckett, C. S.; Dynlacht, B. D.; El-Deiry, W. S.; Elrod, J. W.; Fimia, G. M.; Fulda, S.; García-Sáez, A. J.; Garg, A. D.; Garrido, C.; Gavathiotis, E.; Golstein, P.; Gottlieb, E.; Green, D. R.; Greene, L. A.; Gronemeyer, H.; Gross, A.; Hajnoczky, G.; Hardwick, J. M.; Harris, I. S.; Hengartner, M. O.; Hetz, C.; Ichijo, H.; Jäättelä, M.; Joseph, B.; Jost, P. J.; Juin, P. P.; Kaiser, W. J.; Karin, M.; Kaufmann, T.; Kepp, O.; Kimchi, A.; Kitsis, R. N.; Klionsky, D. J.; Knight, R. A.; Kumar, S.; Lee, S. W.; Lemasters, J. J.; Levine, B.; Linkermann, A.; Lipton, S. A.; Lockshin, R. A.; López-Otín, C.; Lowe, S. W.; Luedde, T.; Lugli, E.; MacFarlane, M.; Madeo, F.; Malewicz, M.; Malorni, W.; Manic, G.; Marine, J.-C.; Martin, S. J.; Martinou, J.-C.; Medema, J. P.; Mehlen, P.; Meier, P.; Melino, S.; Miao, E. A.; Molkentin, J. D.; Moll, U. M.; Muñoz-Pinedo, C.; Nagata, S.; Nuñez, G.; Oberst, A.; Oren, M.; Overholtzer, M.; Pagano, M.; Panaretakis, T.; Pasparakis, M.; Penninger, J. M.; Pereira, D. M.; Pervaiz, S.; Peter, M. E.; Piacentini, M.; Pinton, P.; Prehn, J. H. M.; Puthalakath, H.; Rabinovich, G. A.; Rehm, M.; Rizzuto, R.; Rodrigues, C. M. P.; Rubinsztein, D. C.; Rudel, T.; Ryan, K. M.; Sayan, E.; Scorrano, L.; Shao, F.; Shi, Y.; Silke, J.; Simon, H.-U.; Sistigu, A.; Stockwell, B. R.; Strasser, A.; Szabadkai, G.; Tait, S. W. G.; Tang, D.; Tavernarakis, N.; Thorburn, A.; Tsujimoto, Y.; Turk, B.; Vanden Berghe, T.; Vandenabeele, P.; Vander Heiden, M. G.; Villunger, A.; Virgin, H. W.; Vousden, K. H.; Vucic, D.; Wagner, E. F.; Walczak, H.; Wallach, D.; Wang, Y.; Wells, J. A.; Wood, W.; Yuan, J.; Zakeri, Z.; Zhivotovsky, B.; Zitvogel, L.; Melino, G.; Kroemer, G., Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death & Differentiation* **2018,** *25* (3), 486-541.
11. Christidi, E.; Brunham, L. R., Regulated cell death pathways in doxorubicin-induced cardiotoxicity. *Cell Death & Disease* **2021,** *12* (4), 339.
12. Yu, X.; Ruan, Y.; Huang, X.; Dou, L.; Lan, M.; Cui, J.; Chen, B.; Gong, H.; Wang, Q.; Yan, M.; Sun, S.; Qiu, Q.; Zhang, X.; Man, Y.; Tang, W.; Li, J.; Shen, T., Dexrazoxane ameliorates doxorubicin-induced cardiotoxicity by inhibiting both apoptosis and necroptosis in cardiomyocytes. *Biochemical and biophysical research communications* **2020,** *523* (1), 140-146.
13. Erdogmus Ozgen, Z.; Erdinc, M.; Kelle, I.; Erdinc, L.; Nergiz, Y., Protective effects of necrostatin-1 on doxorubicin-induced cardiotoxicity in rat heart. *Hum Exp Toxicol* **2022,** *41*, 9603271211066066.
14. Kaczmarek, A.; Vandenabeele, P.; Krysko, D. V., Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. *Immunity* **2013,** *38* (2), 209-23.
15. Chan, J. K.; Roth, J.; Oppenheim, J. J.; Tracey, K. J.; Vogl, T.; Feldmann, M.; Horwood, N.; Nanchahal, J., Alarmins: awaiting a clinical response. *The Journal of clinical investigation* **2012,** *122* (8), 2711-9.
16. Satish, S.; Philipose, H.; Rosales, M. A. B.; Saint-Geniez, M., Pharmaceutical Induction of PGC-1α Promotes Retinal Pigment Epithelial Cell Metabolism and Protects against Oxidative Damage. *Oxidative medicine and cellular longevity* **2018,** *2018*, 9248640.
17. Dinulovic, I.; Furrer, R.; Di Fulvio, S.; Ferry, A.; Beer, M.; Handschin, C., PGC-1α modulates necrosis, inflammatory response, and fibrotic tissue formation in injured skeletal muscle. *Skeletal Muscle* **2016,** *6* (1), 38.

18. Rius-Pérez, S.; Torres-Cuevas, I.; Millán, I.; Ortega Á, L.; Pérez, S., PGC-1α, Inflammation, and Oxidative Stress: An Integrative View in Metabolism. *Oxidative medicine and cellular longevity* **2020,** *2020*, 1452696.
19. Liang, D.; Zhuo, Y.; Guo, Z.; He, L.; Wang, X.; He, Y.; Li, L.; Dai, H., SIRT1/PGC-1 pathway activation triggers autophagy/mitophagy and attenuates oxidative damage in intestinal epithelial cells. *Biochimie* **2020,** *170*, 10-20.
20. Peres Diaz, L. S.; Schuman, M. L.; Aisicovich, M.; Toblli, J. E.; Pirola, C. J.; Landa, M. S.; García, S. I., Short-term doxorubicin cardiotoxic effects: involvement of cardiac Thyrotropin Releasing Hormone system. *Life sciences* **2020,** *261*, 118346.
21. Sun, J.; Li, J. Y.; Zhang, L. Q.; Li, D. Y.; Wu, J. Y.; Gao, S. J.; Liu, D. Q.; Zhou, Y. Q.; Mei, W., Nrf2 Activation Attenuates Chronic Constriction Injury-Induced Neuropathic Pain via Induction of PGC-1α-Mediated Mitochondrial Biogenesis in the Spinal Cord. *Oxidative medicine and cellular longevity* **2021,** *2021*, 9577874.
22. Xu, Y.; Kabba, J. A.; Ruan, W.; Wang, Y.; Zhao, S.; Song, X.; Zhang, L.; Li, J.; Pang, T., The PGC-1α Activator ZLN005 Ameliorates Ischemia-Induced Neuronal Injury In Vitro and In Vivo. *Cellular and molecular neurobiology* **2018,** *38* (4), 929-939.
23. Wallace, K. B.; Sardão, V. A.; Oliveira, P. J., Mitochondrial Determinants of Doxorubicin-Induced Cardiomyopathy. *Circ Res* **2020,** *126* (7), 926-941.
24. Osataphan, N.; Phrommintikul, A.; Chattipakorn, S. C.; Chattipakorn, N., Effects of doxorubicin-induced cardiotoxicity on cardiac mitochondrial dynamics and mitochondrial function: Insights for future interventions. *Journal of cellular and molecular medicine* **2020,** *24* (12), 6534-6557.
25. Liu, Y.; Bai, H.; Guo, F.; Thai, P. N.; Luo, X.; Zhang, P.; Yang, C.; Feng, X.; Zhu, D.; Guo, J.; Liang, P.; Xu, Z.; Yang, H.; Lu, X., PGC-1α activator ZLN005 promotes maturation of cardiomyocytes derived from human embryonic stem cells. *Aging* **2020,** *12* (8), 7411-7430.
26. Zhou, Q.; Xu, H.; Yan, L.; Ye, L.; Zhang, X.; Tan, B.; Yi, Q.; Tian, J.; Zhu, J., PGC-1α promotes mitochondrial respiration and biogenesis during the differentiation of hiPSCs into cardiomyocytes. *Genes & diseases* **2021,** *8* (6), 891-906.
27. Brainard, R. E.; Facundo, H. T., Cardiac hypertrophy drives PGC-1α suppression associated with enhanced O-glycosylation. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **2021,** *1867* (5), 166080.
28. Zhu, P.; Ma, H.; Cui, S.; Zhou, X.; Xu, W.; Yu, J.; Li, J., ZLN005 Alleviates In Vivo and In Vitro Renal Fibrosis via PGC-1α-Mediated Mitochondrial Homeostasis. *Pharmaceuticals (Basel)* **2022,** *15* (4).
29. Shi, Z.; Wang, S.; Deng, J.; Gong, Z., PGC-1α attenuates the oxidative stress-induced impaired osteogenesis and angiogenesis regulation effects of mesenchymal stem cells in the presence of diabetic serum. *Biochemistry and biophysics reports* **2021,** *27*, 101070.
30. Valle, I.; Alvarez-Barrientos, A.; Arza, E.; Lamas, S.; Monsalve, M., PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. *Cardiovasc Res* **2005,** *66* (3), 562-73.
31. Di Minno, A.; Turnu, L.; Porro, B.; Squellerio, I.; Cavalca, V.; Tremoli, E.; Di Minno, M. N., 8-Hydroxy-2-Deoxyguanosine Levels and Cardiovascular Disease: A Systematic Review and Meta-Analysis of the Literature. *Antioxidants & redox signaling* **2016,** *24* (10), 548-55.
32. Thomas, M. C.; Woodward, M.; Li, Q.; Pickering, R.; Tikellis, C.; Poulter, N.; Cooper, M. E.; Marre, M.; Zoungas, S.; Chalmers, J., Relationship Between Plasma 8‐OH‐Deoxyguanosine and Cardiovascular Disease and Survival in Type 2 Diabetes Mellitus: Results From the ADVANCE Trial. **2018,** *7* (13), e008226.
33. Qin, F.; Lennon-Edwards, S.; Lancel, S.; Biolo, A.; Siwik, D. A.; Pimentel, D. R.; Dorn, G. W.; Kang, Y. J.; Colucci, W. S., Cardiac-specific overexpression of catalase identifies hydrogen peroxide-dependent and -independent phases of myocardial remodeling and prevents the progression to overt heart failure in G(alpha)q-overexpressing transgenic mice. *Circulation. Heart failure* **2010,** *3* (2), 306-13.
34. Li, X.; Lin, Y.; Wang, S.; Zhou, S.; Ju, J.; Wang, X.; Chen, Y.; Xia, M., Extracellular Superoxide Dismutase Is Associated With Left Ventricular Geometry and Heart Failure in Patients With Cardiovascular Disease. **2020,** *9* (15), e016862.
35. van Deel, E. D.; Lu, Z.; Xu, X.; Zhu, G.; Hu, X.; Oury, T. D.; Bache, R. J.; Duncker, D. J.; Chen, Y., Extracellular superoxide dismutase protects the heart against oxidative stress and hypertrophy after myocardial infarction. *Free radical biology & medicine* **2008,** *44* (7), 1305-13.
36. Sorci, G.; Riuzzi, F.; Agneletti, A. L.; Marchetti, C.; Donato, R., S100B inhibits myogenic differentiation and myotube formation in a RAGE-independent manner. *Molecular and cellular biology* **2003,** *23* (14), 4870-81.
37. Tubaro, C.; Arcuri, C.; Giambanco, I.; Donato, R., S100B protein in myoblasts modulates myogenic differentiation via NF-kappaB-dependent inhibition of MyoD expression. *Journal of cellular physiology* **2010,** *223* (1), 270-82.
38. Tubaro, C.; Arcuri, C.; Giambanco, I.; Donato, R., S100B in myoblasts regulates the transition from activation to quiescence and from quiescence to activation and reduces apoptosis. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **2011,** *1813* (5), 1092-1104.
39. Andrassy, M.; Volz, H. C.; Igwe, J. C.; Funke, B.; Eichberger, S. N.; Kaya, Z.; Buss, S.; Autschbach, F.; Pleger, S. T.; Lukic, I. K.; Bea, F.; Hardt, S. E.; Humpert, P. M.; Bianchi, M. E.; Mairbäurl, H.; Nawroth, P. P.; Remppis, A.; Katus, H. A.; Bierhaus, A., High-Mobility Group Box-1 in Ischemia-Reperfusion Injury of the Heart. **2008,** *117* (25), 3216-3226.
40. Yao, Y.; Xu, X.; Zhang, G.; Zhang, Y.; Qian, W.; Rui, T., Role of HMGB1 in doxorubicin-induced myocardial apoptosis and its regulation pathway. *Basic research in cardiology* **2012,** *107* (3), 267.
41. Bosire, R.; Fadel, L.; Mocsár, G.; Nánási, P.; Sen, P.; Sharma, A. K.; Naseem, M. U.; Kovács, A.; Kugel, J.; Kroemer, G.; Vámosi, G.; Szabó, G., Doxorubicin impacts chromatin binding of HMGB1, Histone H1 and retinoic acid receptor. *Scientific reports* **2022,** *12* (1), 8087.

42. Liu, C.; Cai, Z.; Hu, T.; Yao, Q.; Zhang, L., Cathepsin B aggravated doxorubicin-induced myocardial injury via NF-κB signalling. *Molecular medicine reports* **2020,** *22* (6), 4848-4856.
43. Wu, Q. Q.; Xu, M.; Yuan, Y.; Li, F. F.; Yang, Z.; Liu, Y.; Zhou, M. Q.; Bian, Z. Y.; Deng, W.; Gao, L.; Li, H.; Tang, Q. Z., Cathepsin B deficiency attenuates cardiac remodeling in response to pressure overload via TNF-α/ASK1/JNK pathway. *American journal of physiology. Heart and circulatory physiology* **2015,** *308* (9), H1143-54.
44. Liu, C.; Yao, Q.; Hu, T.; Cai, Z.; Xie, Q.; Zhao, J.; Yuan, Y.; Ni, J.; Wu, Q. Q., Cathepsin B deteriorates diabetic cardiomyopathy induced by streptozotocin via promoting NLRP3-mediated pyroptosis. *Molecular therapy. Nucleic acids* **2022,** *30*, 198-207.
45. Valiente-Alandi, I.; Potter, S. J.; Salvador, A. M.; Schafer, A. E.; Schips, T.; Carrillo-Salinas, F.; Gibson, A. M.; Nieman, M. L.; Perkins, C.; Sargent, M. A.; Huo, J.; Lorenz, J. N.; DeFalco, T.; Molkentin, J. D.; Alcaide, P.; Blaxall, B. C., Inhibiting Fibronectin Attenuates Fibrosis and Improves Cardiac Function in a Model of Heart Failure. *Circulation* **2018,** *138* (12), 1236-1252.
46. Querejeta, R.; López, B.; González, A.; Sánchez, E.; Larman, M.; Ubago, J. L. M.; Díez, J., Increased Collagen Type I Synthesis in Patients With Heart Failure of Hypertensive Origin. **2004,** *110* (10), 1263-1268.
47. Wei, S.; Chow, L. T. C.; Shum, I. O. L.; Qin, L.; Sanderson, J. E., Left and right ventricular collagen type I/III ratios and remodeling post-myocardial infarction. *Journal of Cardiac Failure* **1999,** *5* (2), 117-126.

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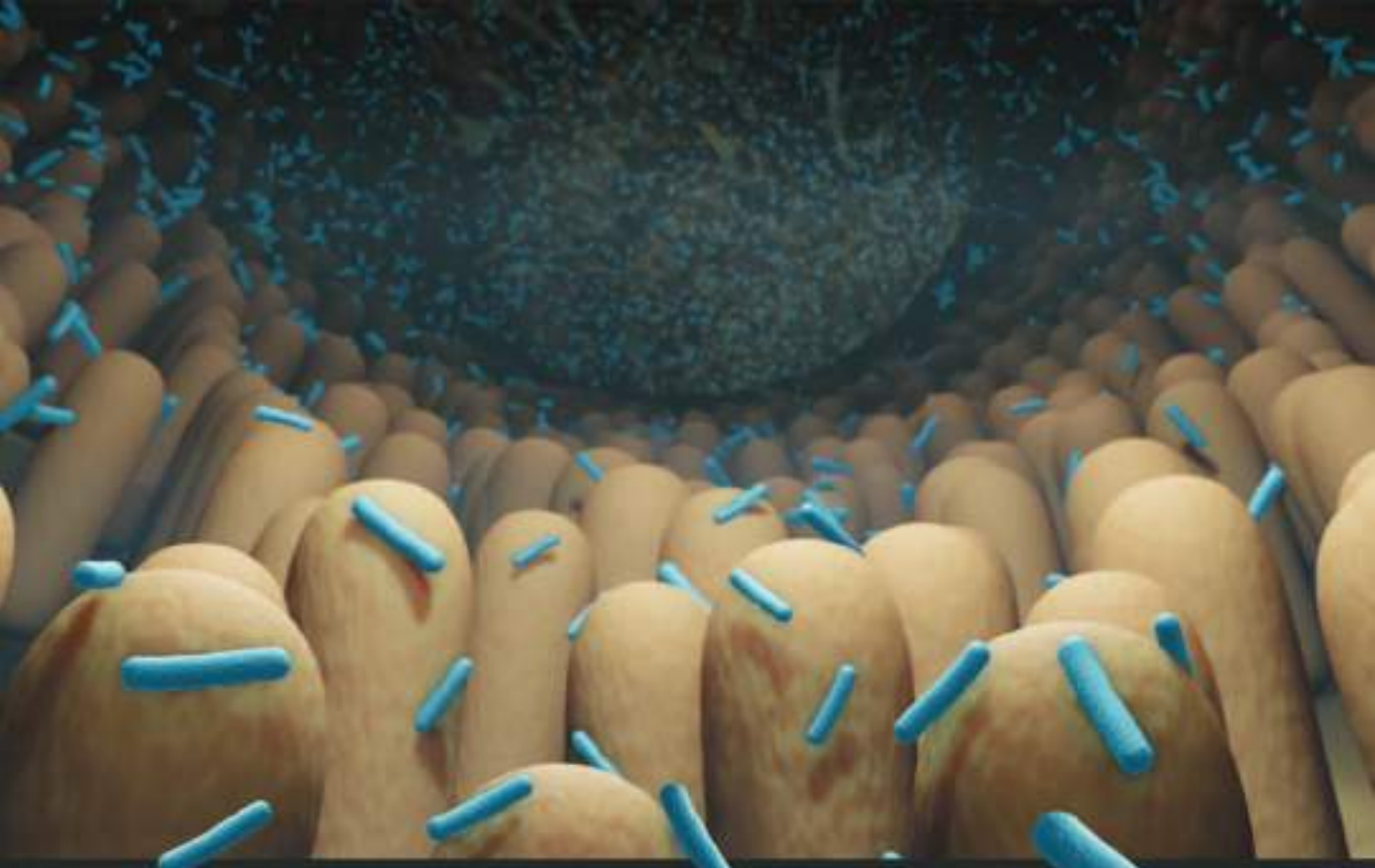

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CHAPTER

4

The modulatory approaches of microbiome therapeutics

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1. Introduction

Microorganisms are the most abundant and the oldest life forms on planet earth. They coevolve with other life forms on earth and remain evolutionarily conserved, which is attributed to their ability to adapt to a wide range of environmental conditions and habitats, helping them to sustain any adverse environmental fluctuations over years. Microorganisms not only dwell amid the harshest environment but also associate with many living organisms as symbionts, parasites, or commensals (Yadav et al., 2021). Humans are no exception. The microbiota has lived in association with humans for centuries, and little was known about the host–microbiota interaction (Gupta et al., 2017; Sharma et al., 2021). Since the beginning of the 21st century, human microbiota research has gained wide attention (Kumar et al., 2017). With the introduction of next-generation sequencing and system biology approaches, human microbiota research has gained momentum and broadened our understanding of the host–microbiota relationship (Yadav et al., 2021). The term "human microbiome" was introduced in 2001 by Joshua Lederberg (Nobel Laureate).

However, there is no precise definition of the human microbiome. The term "microbiota" refers to all microorganisms living in association with humans (inside-out/outside-in), and the term "microbiome" refers to a consortium of microbes and their genes/genome. The study and characterization of genetic constituents of the human microbiome commonly referred to as metagenomics (Gupta et al., 2021; Verma et al., 2018) paved the way to develop engineered microbes (Ahmed et al., 2018). The human microbiome is a diverse pool of microbiota that is commonly found in varied niches including oral cavity, nasopharyngeal, respiratory, gastrointestinal, urogenital tracts, skin, and other body parts. Recent studies have

Microbiome Therapeutics
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reported their critical roles in a broad spectrum of biological processes such as host immunity, metabolism (Yadav et al., 2020), infection, etc. in a spatiotemporal manner that directly affects the host physiological functions (Chauhan, 2019).

The human microbiome was earlier reckoned to encode around a hundred times more genes compared with the whole human genome but the outcome of various studies indicated the estimates to be around tenfolds, but these estimates may be underrated and it may go beyond these estimates with the progress of HMP. The HMP is a conceptual extension of the Human Genome Project led by various associations (e.g., HMP by National Institutes of Health, United States; Metagenomics of the Human Intestinal Tract (Meta-HIT) project, by European Commission) across the globe to understand the evolutionary diversity and structure of human microbiome (Chauhan et al., 2018) factors affecting their abundance; and its impact on human health and susceptibility to diseases. Here, we focus to define a basic understanding of human microbiota, their site-specific niches, cross-talk between microbes of different niches, and various therapeutic strategies to modify the human microbiome in different disease conditions.

2. Human microbiome-based modulatory approaches

2.1 Probiotics

The concept of probiotics evolved after the discovery of beneficial gut microbiota. The idea of probiotics was first conceived by Elie Metchnkoff, a Russian zoologist and Nobel laureate in the early 20th century (Lederberg and McCray, 2001). He conceptualized the idea that a friendly microbiome in yogurt could be used to replace or modulate the gut microbiota population, an article originally published in "The New Yorker." In the past 2 decades, probiotics emerged as a paradigm shift in modern medicine due to the health benefits conferred by probiotics (Monika et al., 2017). Probiotics are described as "live microorganisms which when administered in adequate amounts confer health benefits to the host," the definition is given with the agreement of the World Health Organization (WHO) and the Food and Agriculture Organization (United Nations) (Hill et al., 2014). Despite extensive research, only a few bacterial strains have proven their potential in terms of safety and efficacy. Considering the diversity and organ-specific niches of the human microbiome, lesser is known about beneficial microbiota. However, with the introduction of high-throughput technologies, understanding of the human microbiome has been significantly developed, and it will expand the scope of probiotics in near future (Yadav et al., 2022). Over the years, the health benefits of probiotics have been widely accepted and supported by scientific evidence across the globe. Due to growing popularity and wide acceptability, probiotics are now commonly available in the form of commercial products, e.g., fortified foods, nutraceuticals, fermented dairy products, beverages, cereals, yogurt, tablets, capsules, etc. The most popular bacterial genus (gram-positive bacteria) that is used widely in probiotic formulation includes *Lactobacilli* (*Lactobacillus rhamnosus, Lactobacillus reuteri*), *Bifidobacteria, Bacillus, Escherichia coli* (*Nissle 1917*), *Enterococci* (*Enterococcus faecium (SF68)*), and *Streptococcus* along with yeasts (*Saccharomyces boulardii*) (DiCerbo et al., 2016). Several factors determine the qualification of microbiota to be used as probiotics, e.g., robust delivery system, tolerance of bacterial species to tolerate the host gastrointestinal tract microenvironment (gastric enzymes, acidic pH, bile salts,

etc.), viability/adaptability (ability to survive and colonize in host gut), etc. (Li et al., 2019b; Yadav et al., 2022). However, these limitations can be overcome by the use of adequate micro-/nanoencapsulation techniques (e.g., liposome entrapment, hydrogel, coacervation, fluidized bed coating, etc.) (Calderón-Oliver, 2022; Centurion et al., 2021; Htwe et al., 2019). Some commonly used bacterial strains that are extensively included in probiotics formulations, their characteristics, and proven benefits are discussed in the following:

1) Lactic acid bacteria (gram-positive, cocci, or bacilli, e.g., *Lactobacillus, Pediococcus, Leuconostoc,* and *Streptococcus*) are known to enhance digestion, assimilation, and absorption of nutrients, hydrolyze harmful compounds, and increase bioavailability and have a role in mediating energy homeostasis.
2) *Bifidobacterium* (gram-positive, anaerobic and non-motile) is the major bacterium in the gastrointestinal tract of humans and animals. They are highly efficient at metabolizing complex carbohydrates and serve an important role in host nutrition and metabolism. Commonly used strains include *Bifidobacterium lactis, Bifidobacterium adolescentis, Bifidobacterium thermophilum, Bifidobacterium animalis, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve,* and *Bifidobacterium infantis.*
3) Yeast (*S. boulardii*) is one of the most widely and successfully used strains to treat gastrointestinal infections. It was previously reported to prevent relapse of inflammatory bowel disease (IBD) and colitis (Guslandi and Alberto, 2003; Guslandi et al., 2000) however, its use is limited in immune-compromised patients.
4) *Faecalibacterium prausnitzii* (*Clostridium* cluster IV) are major butyrate-producing microbes in the gut. Butyrate produced by them has enormous beneficial impacts. Being the primary source of energy for intestinal cells, it serves to strengthen the epithelial cells of the intestine, protect against pathogens, stimulate and strengthen the immune system of the host, and helps the host's body to fight chronic conditions such as cancer.

Probiotics can be exploited as modern therapeutics or as an adjuvant to conventional therapies in the management of a variety of human disease conditions, e.g., inflammatory, autoimmune, and infectious diseases (Kumar et al., 2017; Yadav and Chauhan, 2022). With the advancement in probiotics research, now the focus has been shifted toward engineered probiotics. There are many metabolic diseases (diabetes, phenylketonuria, hyperammonemia, etc.) caused by the deficiency of a particular enzyme or defective nonfunctional enzyme that eventually leads to the accumulation of harmful metabolites or lack of essential byproducts. Designing engineered probiotics-based strategies will overcome the aforementioned issues, and it will aid in the treatment of many metabolic disorders. The engineered *Lactococcus lactis* has been reported to produce intact proinsulin (autoantigen) and interleukin-10 (IL-10) cytokine (biologically active), and it may act as an alternative therapy for type 1 diabetes (T1D) ((Takiishi et al., 2012). Engineered *L. lactis* have shown to secrete GAD65370−575 (a T1D autoantigen) and IL-10, and in combination with low-dose anti-CD3 therapy, it helped in improving the pancreatic inflammation in NOD mice (Robert et al., 2014). Recombinant *L. gasseri* (GLP-1-(1−37)-encoding gene fused with a USP45-LEISS secretion marker and a polyhistidine tag) strain secreted GLP1-(1−37) peptide (glucagon-like peptide (GLP) 1, inducing insulin production), and its administration in mice revealed an increased frequency of insulin-producing cells (Duan et al., 2015). Phenylketonuria (PKU) is a metabolic genetic disorder characterized by the accumulation of

phenylalanine that is caused by a defect in the phenylalanine hydroxylase (PAH) enzyme (catalyzing conversion of phenylalanine into other amino acids). When phenylalanine ammonia lyase (PAL)—encoding gene from *Rhodosporidium toruloides* was overexpressed in *E. coli*, the expression system produced biologically active PAL protein, and administration of PAL was capable of reducing the plasma concentration of phenylalanine in the mouse model of PKU (Sarkissian et al., 1999). Similarly, engineered *Lactobacillus reuteri* expressing PAL gene (from *Anabaena variabilis*) was found efficient in lowering phenylalanine in the blood of the PKU mouse model (Durrer et al., 2017). Probiotics can also be exploited in a metabolic disorder called hyperammonemia, caused by a defect in the enzymes catalyzing the conversion of free ammonia to urea. Oral administration of probiotic *Lactobacillus helveticus* strain NS8 and altered Schaedler flora (8 gut commensals consortium) revealed improvement in the symptoms of hyperammonemia (Luo et al., 2014; Shen et al., 2015). Engineered probiotic strain SYNB1020 was developed from *E. coli* Nissle, 1917 that is capable of converting ammonia into L-arginine, and its administration considerably reduced the blood ammonia levels in the mouse. This strain has moved to phase II clinical trials (Kurtz et al., 2019). Various studies are revealing the potential of microbes (e.g., *Enterococcus faecalis, Eggerthella lenta, Pseudomonas aeruginosa*, engineered *E. coli* (Nissle, 1917) in treating neurodegenerative diseases (Maini Rekdal et al., 2019) and infectious diseases (Saeidi et al., 2011; Sassone-Corsi et al., 2016). The role of probiotics in organ and/or disease-specific conditions is discussed in later sections.

2.2 Prebiotics

Prebiotics was first conceptualized and defined by Roberfroid and Gibson in 1995 as "indigestible (fructans and glucans enriched) dietary fibers (e.g., fructooligosaccharides, galactooligosaccharides, and trans-galactooligosaccharides) that act as substrate and capable of stimulating selective microbiota population, thereby conferring health benefits to host" (Gibson et al., 2017). Over the years, a better understanding of the microbiome and their metabolites has broadened the definition, and it is continuously evolving due to a rapidly expanding list of microbial metabolites (e.g., polyphenols and polyunsaturated fatty acids (PUFAs), short-chain fatty acids (SCFAs)). The term prebiotics should not be muddled with probiotics; the former refers to nonliving substrate/metabolites acted upon by microbes, whereas the latter represents live microbes/modified microbial strains. Since probiotics and prebiotics are complementary to each other, a new modulatory approach developed where both are being used in combination, and it is referred to as "synbiotics" (Schrezenmeir and Vrese, 2001). Among SCFAs, butyrate serves as an energy fuel for colonocytes, whereas muscle tissue and liver utilize acetate and propionate for energy production and gluconeogenesis, respectively. Butyrate functions as an inhibitor for histone deacetylase activity and has been proven to be beneficial in some colonic cancers, leading to a reduction in inflammation and oxidative stress (Mehta et al., 2021) and enhancing barrier protection functions of epithelia in the host (Roberto et al., 2011). Besides the microbiome-based nutritional function of prebiotics, it can also confer direct health benefits and are easily assimilated into the host body, one of the most well-known among which is the antiadhesive action of prebiotics. Interaction between bacterial receptors and prebiotics inhibits them from interacting with epithelial cells,

perturbing their colonization. The lifestyle, genetics, and nutritional factors greatly vary from individual to individual and geographical locations greatly limiting the health benefits of probiotics and prebiotics. In a comparative metagenome and metabolome study involving north-middle and South Indian cohorts, the predominance of "branched-chain amino acid biosynthesis pathways" utilizing *Prevotella* species were observed in the former cohort, whereas the latter cohort demonstrated "branched-chain amino acid transporters and SCFA" biosynthesis pathways utilizing *Bacteroides, Faecalibacterium,* and *Ruminococcus* species (Dhakan et al., 2019). All these factors need to be considered while using probiotics, prebiotics, and related engineered approaches in humans. Like probiotics, prebiotics research also revealed several health benefits in humans that range from cell metabolism, immune regulation, anti-microbicidal (Kumar et al., 2021), antiinflammation, neuroendocrine function, etc. (Belkaid and Hand, 2014; Clarke et al., 2014; Strandwitz, 2018; Martin et al., 2019). All these physiological functions are mediated by secretion of active metabolites, e.g., neurotransmitters (acetylcholine [*Lactobacillus plantarum*], noradrenaline [produced by *Proteus vulgaris, Bacillus mycoides, Bacillus subtilis*], gamma-aminobutyric acid [*Bifidobacterium* and *Lactobacillus*]), histamine (*S. thermophilus, Lactobacillus spp., Lactococcus*), serotonin (*Lactobacillus spp., Lactococcus, E. coli, Klebsiella, S. thermophilus*) (Strandwitz, 2018), and water-soluble vitamins (*Bifidobacterium, Bacteroides,* and *Enterococcus)* (Said and Nexo, 2018). Many gut microbial species (Clostridiaceae family, *Lactobacillus casei, Lactobacillus paracasei, Bifidobacterium adolescentis, Alcaligenaceae* family members and *Achromobacter xylosoxidans*) have demonstrated the ability to elicit immune responses by activating the immune cells (T helper cells, regulatory T cells, dendritic cells, etc.) to produce effector molecules, e.g., cytokines (IL-6, IL-17, IL-22, etc.) and antibodies (IgA) in cell/tissue-specific manner (Fung et al., 2016; Gaboriau-Routhiau et al., 2009; Galdeano and Perdigón, 2006; Tan et al., 2016; Tsai et al., 2010). The inulin-like dietary fructans (oligofructose and synergy 1) induce the production of proglucagon and GLP-1 in the intestine that was found to be critical in regulating body glucose and weight (Cani et al., 2004), but the precise mechanism of action of the prebiotics is not known. All these bacteria can be engineered to efficiently produce the prebiotic metabolites in a targeted manner, and future research should be focused on this direction.

2.3 Postbiotics and parabiotics

The probiotics research revealed that nonviable microbes and their cell-free extracts/by-products are equally important with proven health benefits (immunomodulatory, antiinflammatory, antioxidant activities, etc.), and this notion has led to the emergence of the concept of postbiotics (a term proposed by Tsilingiri et al.) and parabiotics (Tsilingiri and Rescigno, 2013). However, there was no consensus on the precise definition of postbiotics, but in 2019, Salminen et al. proposed a definition in International Scientific Association for Probiotics and Prebiotics (ISAPP) (Salminen et al., 2021). The expert panel defined the postbiotics as "preparation of inanimate microorganisms and/or their components that confers a health benefit to the host" to bring agreement and uniformity among the researchers working in the field. Therefore, postbiotics should effectively contain killed/inactivated microorganisms or their cell components/metabolites with evident health benefits to the recipient host. Postbiotics are relatively safer compared with probiotics that employ live microorganisms. The postbiotics protein *P*40 and *P*75 were isolated from the cell wall of *L. rhamnosus, L. casei,*

and *L. paracasei,* and they were reported to possess immunomodulatory function and epidermal growth factor receptor function and inhibit apoptosis, inflammation, and other regulatory proteins (Bäuerl et al., 2019). Aggregation-promoting factors (APFs) secreted from *Lactobacillus sp.* (e.g., *L. gasseri SBT2055, Lactobacillus delbr, L. gasseri ATCC 9857*) are another class of postbiotics reported to increase host colonization and excluding the pathogens (e.g., *Campylobacter jejuni, Trichomonas vaginalis*) by decreasing their adhesion and invasion (Nishiyama et al., 2014; Phukan et al., 2018; Yungareva and Urshev, 2018). The antimicrobial peptides, e.g., bacteriocins (*Lactobacillus acidophilus* and *Lactobacillus johnsonii*), lactocin (*Lactobacillus casei*), lactoccin G (*Lactobacillus lactis),* and plantaricin (*Lactobacillus plantarum*) (Nielsen et al., 2010; Perez et al., 2014; Zacharof and Lovitt, 2012), were reported to mediate potent inhibitory effects on pathogens, and they can serve as an alternative to antibiotics without developing the risk of antibiotic resistance (Ahmed et al., 2013, 2018) and other deleterious effects (Chauhan et al., 2017). There are various other small molecule—based postbiotics such as SCFAs, conjugated linoleic acid, and neurotransmitters derived from a myriad of the human microbiota; the role of some of which in human health and diseases has been already discussed in previous sections and some will be addressed in later sections. Compared with postbiotics that include mainly the secreted microbial components, the parabiotics are mainly derived from microbial cell wall components, e.g., peptidoglycan, teichoic acid, polysaccharides, cell surface proteins, LPXTG protein, S-layer protein, pili proteins, moonlighting proteins (e.g., molecular chaperones, metabolic enzymes, elongation factors, and ribosomal proteins) that play a critical role such as the colonization of commensals in host ecosystem and/or inhibiting the adhesion, invasion, and proliferation of pathogenic microbes besides regulating host immunity.

2.4 Fecal microbiota transplantation

The fecal microbiota transplantation (FMT) procedure involves the introduction of a solution of fecal matter (prepared in sterile water or saline followed by removal of insoluble particulate matter by filtration) from the healthy donor (with no associated infectious, autoimmune, metabolic, malignant, or inflammatory diseases) into the colon of the recipient via endoscopic procedures (esophago-gastro-duodenoscopy, nasogastric tube, nasojejunal, nasoduodenal [upper gastrointestinal (GI) route], colonoscopy [lower GI route]), retention enema (lower GI route), or oral capsules (modified approach). This microbial transplantation technique can change the microbial composition of the recipient and is capable of conferring health benefits by displacement of the pathogenic microbes. The FMT is having the historical background of around 3000 years when cow feces were used as therapy/enemas for gastrointestinal ailments in ancient India. In China, around the 4th century ago, the use of a mixture of feces and water (yellow soup) in the treatment of diarrhea and food poisoning has been described by Ge Hong (Zhang et al., 2012). FMT has been introduced in modern medicine when fecal enema was used as an adjunct in pseudomembranous enterocolitis treatment (Eiseman et al., 1958). Later the FMT application was extended to noninfectious diseases where bowel flora alteration has been suggested as a potential treatment option in inflammatory bowel disease (Borody et al., 1989). With extensive clinical trials, at present, FMT has been established as a treatment option in recurring *Clostridium difficile* infection (van Nood

et al., 2013; Smits et al., 2013). With the emerging health benefits of the human microbiome and their implication in human physiology and disease, FMT has started gaining acceptance worldwide owing to its advantages of curing dysbiosis without any potential harm, which is commonly associated with conventional therapies (Mittal et al., 2019). However, FMT is associated with considerable risks that range from minor symptoms (borborygmus, diarrhea, nausea, vomiting, flatulence, transient fever) to severe complications (perforation, bleeding, aspiration, peritonitis, sepsis, the transmission of pathogens like Hepatitis C, HIV, etc.). Considering the associated risk, several regulatory norms were employed worldwide to use FMT as treatment. US-FDA regarded FMT as a biological product that can be used as a drug to diagnose and treat human diseases, whereas FMT was considered as a "new biologic drug" by Health Canada and standard safety norms of the clinical trial were proposed (Allen-Vercoe et al., 2012; Center for Biologics Evaluation and Research, 2016). There are many FMT-based modulatory approaches such as oral capsules (noninvasive and safest route of administration) that were developed by various pharmaceutical companies, e.g., Seres Therapeutics (capsule containing microbial spore), Maatpharma (restorative therapies using autologous microbiome); Finch therapeutics (capsules with allogenic microbiota); Vedanta (capsules containing bacterial consortia) (Yadav and Chauhan, 2022). The future roadmap of FMT (from "filthy poop" to "modern medicine") depends on the understanding of the gut microbiome and the mechanism of action of the microbiome in context with host health (Yadav and Chauhan, 2020); FMT may be used in a targeted manner to enrich a particular consortium of microbiota that can be used in combination with probiotics, prebiotics, or postbiotics to enhance the clinical outcome of FMT. The implication of FMT-based therapies (species enrichment modulatory approaches) in different human diseases has been described in successive sections.

2.5 Phage-mediated therapy

Phage-based modulation of the human microbiome is one of the most powerful tools owing to their ability to infect (lytic and lysogenic), self-replication, high specificity, and ubiquitous nature that makes them to easily infect their target microbes and aid in their selective clearance (Yadav and Chauhan, 2020). Phages, e.g., bacteriophages are promising alternatives for traditional antibiotics therapy especially in targeting antibiotic-resistant pathogens/bacteria. There were various in vivo studies carried out in mouse models that demonstrated the role of bacteriophages in significantly ameliorating human diseases. In a humanized mouse model of alcoholic liver disease, a cocktail of four phages was used to target the cytolytic *E. faecalis* and it improved the symptoms (Duan et al., 2019). Bacteriophage-based therapies can be combined with other microbiome-based treatment approaches such as FMT or probiotics. In a similar combinatorial approach, when sterile fecal microbiota (FMT-based isolation) enriched with phages (pathogen targeted) from healthy donors was transplanted to recurrent *C. difficile* patients, the infection-associated symptoms were relieved (Ott et al., 2017). Fecal virome transplantation from lean to obese mouse model revealed a significant reduction in type 2 diabetes and obesity symptoms (Rasmussen et al., 2020). We have discussed the bacteriophage-mediated microbiome modulatory approaches in various organ-specific diseases in successive sections.

3. Microbiome consortia: organ-specific distribution in the human body

The human body acts as a unique ecosystem, and the human microbiome is ubiquitously distributed on and inside various organs, and their diversity greatly varies depending on the specific niche as mentioned before. The human microbiome is considered an individual organ as the total human microbiota components in a healthy adult individual account for around 1.5−2 kg of body weight (Baquero and Nombela, 2012; Pflughoeft and Versalovic, 2012; Qin et al., 2010), and majority of the microbiota resides in gastrointestinal tract, particularly small and large intestine. In terms of numbers, the human microbiota (100 trillion) surpasses the total number of human cell counts (~ 10 trillion) by a 10:1 proportion. Furthermore, the total number of genes in human was estimated to be around 23,000, and microbial genes (~ 3.3 million) account to be ~ 150-fold more than human genes (Qin et al., 2010). In terms of microbiota density, the colon contains the highest, i.e., 10^{14} microbes followed by the skin, i.e., 10^{12} microbes (Berg, 1996; Sender et al., 2016).

The metagenomic study reported that the gut microbiota acts as blueprint of an individual, out of the 1000 bacterial species identified in the gut, only 150 species are common between the individuals, and the remaining are host-specific (Qin et al., 2010; Kumar et al., 2016). This unique blueprint signature of the gut microbiome is fully developed by the age of 3 years (Qin et al., 2010; Raveh-Sadka et al., 2015). The gut microbiome diversity is greatly influenced by host lifestyle (dietary intake), aging, and environmental factors (Dominguez-Bello et al., 2010; Yatsunenko et al., 2012; Zhong et al., 2019). Indian population having predominant plant-based diet (North-Middle India cohort) showed *Prevotella* species (using branched-chain amino acid and lipopolysaccharide dominant biosynthetic pathway)−enriched signature, whereas omnivorous cohort of south India revealed *Bacteroides*, *Faecalibacterium*, and *Ruminococcus* species (branched-chain amino acid transporters and SCFA biosynthesis pathways)−enriched signature in the gut (Dhakan et al., 2019). As mentioned before, there are growing shreds of evidence demonstrating the immense impact of human microbiota and their metabolites in maintaining the fine-tuning with various human physiological processes in a symbiotic manner, and dysbiosis is associated with several diseases (discussed in late sections). In this section, we will discuss the major organ niche-specific human microbiome, their role in organ homeostasis, and diseases to understand the fundamentals of "eubiosis" and "dysbiosis."

3.1 Oral microbiome and modulatory approaches

The oral cavity comprises the anterior oral fissure (lips opening) and posterior oropharyngeal isthmus (oropharynx opening) that mainly include lips, hard and soft palate, buccal mucosa (lips/cheeks inner lining), tongue, gums (gingiva), mouth floor (beneath the tongue), and retromolar trigone. The collection of microbiota and their genome present in the oral cavity is known as oral microbiome. Like other organs, oral microbiome is also thought to be colonized in the oral cavity during pregnancy to birth. The first evidence of an oral microbiome was found in 1674 when Antony van Leeuwenhoek observed small animalcules in his dental plaque with a self-designed microscope (Shankargouda et al., 2013). The oral microbiome is considered the second largest and most highly diverse microbial community

after the gut microbiome. The Human Oral Microbiome Database (HOMD) launched in 2010 revealed over 600 (619 taxa and 13 phyla based on 16S rRNA gene sequencing) bacterial genera in the oral cavity, and out of these, only 50% have been cultivated (Dewhirst et al., 2010). The HOMD is now termed expanded HOMD (eHOMD) that includes 775 genera, out of which only 70% can be cultivated (57% were named officially, and 13% were unnamed) and 30% were uncultivated. However, host interaction with environmental factors including lifestyle (e.g., diet, tobacco, alcohol, drugs, stress, etc. (Kumar et al., 2020)) largely affects its microbiome homeostasis. The 16S rRNA gene NGS (next-generation sequencing) technique is comparatively most advanced than conventional culture-based methods to study the human microbiome, and it provides deeper insights into microbial diversity. Studies based on 16S rRNA gene NGS revealed the common occupancy of *Actinomyces, Corynebacterium, Capnocytophaga, Fusobacterium, Granulicatella, Haemophilus, Neisseria, Prevotella, Porphyromonas, Rothia,* and *Streptococcus* (Caselli et al., 2020; Jørn et al., 2005) bacterial species in the oral cavity; however, site-specific variation in the bacterial genera was found (Segata et al., 2012). Segata et al. reported the presence of bacterial species of *Porphyromonadaceae, Veillonellaceae,* and *Lachnospiraceae* family that are common to most oral sites (tongue, tonsils, gingival [sub/supra] plaques, hard palate, buccal mucosa, keratinized gingiva, saliva, throat) of the healthy oral microbiome (HOM), but their distribution varies in different oral sites. The major limitation associated with most studies is that they mainly provide an insight into oral bacteriome rather than a complete oral microbiome that may extend to fungi, protists, and/or viruses. To address this fact, Caselli et al. performed whole-genome sequencing (WGS) and resistome (collection of all antibiotic resistance genes of the human microbiome) analysis of the healthy oral microbiome and identified over 200 genera in healthy oral microbiota (Caselli et al., 2020). Furthermore, no significant interindividual difference was observed in HOM supporting the concept of core HOM (the human microbiome has two components: a core microbiome that remains common in all individuals; and a variable microbiome that alters between individuals based on lifestyle and other host-environmental factors). In oral mucosa (oral mucosa, hard palate, and keratinized gingiva), *Streptococci* were most abundant ($>50\%$), whereas they were less abundant (up to 23%) in other oral sites (saliva, tongue, oral rinse, and gingival plaque) (Caselli et al., 2020). The study also detected the presence of fungi (mycome) that were limited to oral rinse, hard palate, and supragingival plaque (mainly *Candida* with *Saccharomycetales,* 0.004% of total HOM), but no protozoa were detected (may be due to the small sample size or due to subject selection). However, viruses (virome) are relatively more abundant than fungi accounting for around 0.03% of HOM, mainly limited to supragingival plaque and mucosal sites, including viruses of family *Herpesviridae* phages, phage K13 (*Streptococcus*), S1249 (*Aggregatibacter*), phage targeting bacteria of families, e.g., *Myoviridae, Siphoviridae, Podoviridae, Haemophilus,* etc. (Dhakan et al., 2019). The resistome data of HOM is limited; resistance genes conferring resistance toward tetracycline, macrolides, lincosamides, quinolones, and streptogramin were identified, but the strain-specific origin of resistance genes was not determined (Segata et al., 2012). The oral microbiome resides in the oral cavity as commensals and shares a symbiotic relationship with the host. These commensals form a protective shield, and disease is developed only when the barrier is breached by pathogenic microbes. The oral bacteria exert their effect on various other host organs, e.g., oral bacteria with nitrate-reductase activity catalyze dietary nitrate conversion to nitrites, which is further converted into nitric oxide (NO). Nitrite induces

gastric mucus secretion; nitrate inhibits platelet function, lowers blood pressure, and decreases endothelial dysfunction; and NO is a potent vasodilator, supporting the notion of oral–gut–heart axis functioning (Kapil et al., 2010; Lundberg et al., 2009, 2011; Velmurugan et al., 2016). Therefore, HOM is critical in maintaining the general physiology of the host, and their association has been reported with various oral diseases such as caries, gingivitis, periodontitis, etc. under oral dysbiosis condition that is beyond the scope of this chapter.

There are various clinical trials performed that demonstrated the clinical relevance of microbiota as probiotics in various oral conditions, but contradictory reports are also available and skepticism remains over the cautious use of probiotics. Here, we will discuss some distinct nonconventional strategies other than probiotics to combat pathogenic microbes. A recent phase III clinical trial study, where SER-109 (an oral microbiome) composed of purified spores of *Firmicutes*, was used in the treatment of recurrent *Clostridioides difficile* infection (Feuerstadt et al., 2022). In the double-blind, randomized, placebo-controlled trial study, superior efficacy was observed in the SER-109 oral administration group compared with the placebo group. Dassi et al. have shown the short-term effect of probiotics (enriched in *L. delbrueckii subsp., S. thermophilus*, *Bulgaricus*, and *L. paracasei* strains) on oral microbiome diversity; however, the impact of other constituents (e.g., vitamins B6 and D) of the probiotic formulation cannot be ruled out in maintaining the microbiota diversity (Dassi et al., 2018). An observer-blind, parallel, randomized clinical trial reported the ability of probiotics assimilation into dental biofilms (Arweiler et al., 2020), indicating the modulatory effect of commercial probiotics on oral microbiota diversity, but it did not significantly alter the oral microbiome structure. Furthermore, urea can act as "prebiotics," and it may be used as anticaries therapy. Urea (present in healthy saliva at the concentration of 3–10 mmol/L urea) and arginine (present in parotid saliva at concentrations of $\sim$ 50 μmol/L) act as metabolites that are converted by few oral bacteria (e.g., *Actinomyces naes-lundii, Haemophilus spp.,* and *S. salivarius* possess urease activity, whereas arginine metabolized by deiminase system (ADS) of bacteria *Streptococcus sanguinis, Streptococcus parasanguinis, Streptococcus gordonii, Streptococcus mitis, Lactobacilli,* and *Actinomyces* strains) into ammonia to maintain the alkaline milieu in the oral cavity (Liu et al., 2012; Nascimento, 2018). However, urea as prebiotic supplementation is not established in dental caries, but studies reported an anticaries effect in children using sugar-free chewing gums having urea compared with the no-gum group (Machiulskiene et al., 2001; Petersen and Razanamihaja, 1999). There are various promising prebiotic compounds, e.g., beta-methyl-D-galactoside, Met-Pro, *N*-acetyl-D-mannosamine, and succinic acid that showed the in vitro potential to promote the growth of oral microbiota, but their use as prebiotics remains to be investigated (Slomka et al., 2018). The use of probiotics has been widely explored in various oral disease conditions where conventional genera, i.e., *Lactobacillus* and *Bifidobacterium* were used in the probiotics supplementation, but contradictory findings were reported. But both these genera are acidogenic/aciduric and may favor dental caries, and their harmful effects may overweigh benefits. Identifying the commensal bacterial strains that occupy the same niche competing with pathogenic strains and producing compounds inhibiting pathogens (e.g., *Streptococcus mutans*) are promising candidates to be used in probiotics. Two such recently discovered strains are *Streptococcus dentisani* and *Streptococcus A12*, which possess unique features such as active colonizers of the oral cavity, possess arginolytic activity (maintain alkaline pH), and produce compounds such as bacteriocins (e.g., *S. dentisani*) and challisin-like protease (e.g., *Streptococcus A12*), thereby inhibiting

the pathogenic *S. mutans* and other cariogenic strains (Huang et al., 2016; López-López et al., 2017; Tanzer et al., 1982). Displacement of cariogenic native *S. mutans* with engineered *S. mutans* (deficient in lactic acid production and polysaccharide metabolism (Kumar et al., 2018)) that have no or low pathogenicity (Hillman et al., 2007) is a promising therapeutic strategy in modulating the oral microbiome. Another strategy is the identification of biosynthetic gene clusters (BGCs) that are associated with oral health and disease development. BGCs are reported to encode compounds or metabolites that may have therapeutic benefits and may contribute to remodeling of oral ecology by inhibiting the growth of cariogenic or pathogenic strains (Aleti et al., 2019; Donia et al., 2014). Besides displacement therapy, the use of antimicrobial peptides (e.g., ZXR-2 and CLP-4) and specifically targeted antimicrobial peptides (STAMPS—designed to have a specific targeting domain and a killing domain) that are synthetic peptides (e.g., C16G2) is equally promising, particularly STAMPS that demonstrated the efficiency to specifically kill the *S. mutans* biofilms rendering the commensal strains unharmed (Chen et al., 2017; Eckert et al., 2006; Guo et al., 2015). Employing small molecules (e.g., 3F1 and quinoxaline derivative) is another strategy that may be used to disrupt the biofilm formation by cariogenic species (*S. mutans*), and there is an enormous list of such small molecules that are yet to be validated for their efficacy (Chen et al., 2017; Saputo et al., 2017; Garcia et al., 2017). Another very attractive strategy is bacteriophage against the cariogenic/pathogenic bacterial species, but it is also associated with a disadvantage that it can eliminate the pathogenic microbes along with commensals and may be detrimental to the entire oral ecosystem, and therefore can be used only in a targeted manner (as engineered phages). Targeting the oral microbiome and/or other human microbiota in developing therapeutics in oral health management is a very exciting area, but the knowledge of HOM is in the nascent stage, and thorough investigation is desired in this area to develop robust oral microbiome-based modulatory approaches.

3.2 Nasal (nasopharyngeal/upper respiratory tract) microbiome

The nasal passage or upper respiratory tract mainly involves nostrils, nasal cavities, sinuses, vestibules, turbinates, the portion of the mouth, pharynx (throat), and larynx (voice box). In this section, we have used the term "nasal microbiome," which is defined as the collection of total microbiota and their genes present in the upper respiratory tract. It is estimated that an average adult breathes (inhales and exhales) 7−8 L of air per minute or 10,000−11,000 L per day, and it contains over 10^4−10^6 microbes (bacteria) per meter cube (m^3) of air inhaled. Hence, the nasal passage is constantly exposed to external environmental factors including air, humidity, pollutants, and pathogens rendering the upper respiratory tract as an immunologically active site. The nasal microbiome is also very diverse in nature, and microbial abundance varies at different sites. In a healthy adult, the anterior nares that open in the nasal cavity demonstrated the presence of four main phyla, i.e., *Firmicutes, Bacteroidetes, Actinobacteria,* and *Proteobacteria* (Bassis et al., 2014), and common genera include *Staphylococcus, Streptococcus, Corynebacterium, Bifidobacterium, Propionibacterium, Haemophilus, Moraxella, and Dolosigranulum* (Bassis et al., 2014; Zhou et al., 2014a). The middle meatus is reported to have bacterial species such as *Staphylococcus aureus, Staphylococcus epidermidis,* and *P. acnes* (Ramakrishnan et al., 2013). The microbial composition of sphenoethmoidal recess and middle meatus is identical, but the microbial diversity was found lesser in the

latter (Yan et al., 2013). The microbiota of the nasopharynx is similar to that of the anterior nares of adults (Stearns et al., 2015). However, the nasal microbiome varies with age, and microbes are detected in the nasopharynx of healthy neonates irrespective of the mode of delivery (i.e., cesarean vs. vaginal) supporting the notion that early colonization occurs much before birth. The diversity and density gradually vary with age; during early life, most common genera, e.g., *Staphylococcus, Streptococcus, Haemophilus, Moraxella, Corynebacterium,* and *Dolosigranulum*, were detected (Biesbroek et al., 2014; Mika et al., 2015). At 1.5 months, *Corynebacterium, Dolosigranulum,* and *Moraxella* were detected in the upper respiratory tract. However, the nasal microbiome of infants is affected by several environmental/lifestyle factors; the breastfeeding infants showed the signatures of *Dolosigranulum* or *Corynebacterium*, whereas the presence of *S. aureus* was observed in formula-fed infants (Biesbroek et al., 2014a,b; Mika et al., 2015). Furthermore, seasonal variation was reported in the nasal microbiome of infants with a surge of *Corynebacterium* in summers and *Pasteurellaceae* in winters (Mika et al., 2015). Up to 2 years of age, the nasal microbiota demonstrated a stable population of *Corynebacterium, Dolosigranulum,* and *Moraxella* (Biesbroek et al., 2014a, b). The changes in infant microbiota composition are also associated with disease susceptibilities; the presence of *Moraxella catarrhalis*, *Haemophilus influenza,* and *S. pneumonia* is associated with wheezing in infants, whereas infants with a predominance of *Moraxella* species were less likely to suffer from upper respiratory infection (Biesbroek et al., 2014a, b). Similarly, the *Streptococcus*-enriched profile of the nasopharyngeal tract in infants serves as an indicator of asthma susceptibility (Teo et al., 2015), and *Haemophilus*-dominant nasopharyngeal microbiota increases the severity of bronchiolitis (Luna et al., 2018). With aging, the nasal microbiota showed alterations, the anterior nares of elderly individuals (>65 years) were found to be similar to the oropharynx of adults, and their oropharynx microbiota reported predominance of *Streptococci* species (mainly *Streptococcus salivarius*) (Lemon et al., 2010). The anterior nares/nostrils of mid-aged individuals (18–40 years) were dominated by *Corynebacterium, Propionibacterium,* and *Staphylococcus*, whereas the oropharynx demonstrated the prevalence of *Staphylococcus, Veillonella,* and *Streptococcus* (Lemon et al., 2010; Whelan et al., 2014). The majority of the healthy nasal microbiome investigations are mainly restricted to bacteriomes, but studies are also available indicating the presence of viruses (bacteriophages, DNA, and RNA viruses) and fungi in the nasal microbiome of healthy and disease conditions (Hogan and Kolter, 2002; Wylie et al., 2014; Zhao et al., 2018). The various microbiome modulatory approaches are discussed in the aforementioned sections; investigations in similar lines have been employed in the nasal microbiome modulations, but probiotics remain the predominant choice in most investigations/trials. The role of probiotics is widely explored as therapy in dysbiosis or various upper respiratory tract diseases; some of the noticeable investigations are summarized in Table 4.1.

3.3 Ocular microbiome

The ocular microbiome refers to microbiota present in/on the human eyes. In recent years, ocular microbiome research captured interest to understand the microbiota of the eye, their role in ocular health, and its association with ocular diseases. A part of an eye is in direct contact with the external environment (ocular surface) and is constantly challenged by pathogenic microbes. Earlier the existence of ocular surface (eyelids, cornea, tear film, and conjunctiva)

TABLE 4.1 Summary of the investigations/trials related to nasal diseases and various microbiome-based modulatory approaches.

S. No.	Disease	Study type (sample size)	Probiotic strain	Dosage (mode of administration)	References	Outcome
1.	Chronic rhinosinusitis (CRS)	Prospective open-label pilot trial (of safety and feasibility) (probiotics, n = 24)	*Lactococcus lactis W136*	Nasal spray (14-day course of BID sinus irrigations containing 1.2×10^9 CFU)	Endam et al. (2020)	Dosage was safe with transient improvement
2.	CRS without nasal polyps	Randomized, double-blind, cross-over, and sham-controlled design (n = 20)	Honeybee lactic acid bacteria (mixture of nine *lactobacilli* and four *bifidobacteria*)	Nasal spray (2-week treatment)	Mårtensson et al. (2017)	No significant improvement
3.	Allergic rhinoconjunctivitis	Parallel double-blind, randomized, placebo-controlled study N = 173 (n = 87, placebo; n = 86, probiotics)	*Lactobacillus gasseri KS-13, Bifidobacterium bifidum G9-1, Bifidobacterium longum MM-2*	Oral (2 capsules/day, 1.5×10^9 CFU/capsules)	Dennis-Wall et al. (2017)	Improvement in rhinoconjunctivitis-specific quality of life
4.	Allergic rhinitis (AR)	Prospective double-blind, randomized, placebo-controlled study, N = 100 (SLIT (sublingual immunotherapy)+ vitamin D, n = 25; SLIT + placebo, n = 25; SLIT + probiotics, n = 25; control, n = 25)	*Lactobacillus rhamnosus GG*	Oral (5-grass SLIT 300 IR tablets with either vitamin D 1000 IU daily supplementation, probiotic, or placebo)	Jerzynska et al. (2016)	Probiotic supplementation demonstrated enhanced clinical and immunological efficacy
5.	AR	Parallel double-blind, randomized, placebo-controlled study N = 158 patients (placebo, n = 20; SIT (allergen-specific immunotherapy, n = 46; Cb (*Clostridium butyricum*), n = 48; n = , SIT/Cb, n = 44)	*C. butyricum*	Oral probiotics (2 capsules of 420 mg twice/day)	Xu et al. (2016)	Cb enhances efficacy of SIT on AR

(*Continued*)

TABLE 4.1 Summary of the investigations/trials related to nasal diseases and various microbiome-based modulatory approaches.—cont'd

S. No.	Disease	Study type (sample size)	Probiotic strain	Dosage (mode of administration)	References	Outcome
6.	Perennial allergic rhinitis (PAR)	Double-blind, randomized, placebo-controlled study (levocetirizine + placebo, n = 28; levocetirizine + probiotics, n = 32)	*Lactobacillus paracasei* (HF.A00232)	Oral (HF.A00232) (5×10^9 CFU/daily)	Lin et al. (2014)	No significant improvement
7.	Seasonal allergic rhinitis	Parallel double-blind, randomized, placebo-controlled study (placebo, n = 10; probiotics, n = 20)	*Bifidobacterium lactis* NCC2818 (4 £ 109 CFU/day)	Oral (~2 g/day, 4×10^9CFU/day)	Singh A et al. (2013)	Mitigates seasonal allergic symptoms
8.	CRS	Prospective, randomized, double-blind, placebo-controlled trial (probiotics, n = 78; placebo (n = 79	*Lactobacillus rhamnosus R0011*	Oral (500 million active cells/tablets, twice/day; for 4 weeks)	Mukerji et al. (2009)	No significant improvement in sinonasal quality-of-life scores for longer period, only transient improvement
9.	PAR	Double-blind, randomized, placebo-controlled study, N = 49 (placebo, n = 24; probiotics, n = 25)	*Lactobacillus acidophilus* L-92	Oral (3×10^{10} CFU/day)	Ishida et al. (2005)	Alleviate the symptoms of PAR
10.	CRS	Double-blind, placebo-controlled multicenter study (probiotics, n = 78; placebo (n = 79	*Enterococcus faecalis* (Symbioflor1)	Oral (3 × 30 drops/day for 6 months; follow-up for 8 months)	Habermann et al. (2002)	Decrease in CRS symptoms

microbiota was debatable since the lacrimal gland secretion consists of lysozyme that is having microbicidal activity. In the past decade, various studies reported the existence of ocular surface microbiota and successfully cultured various bacterial populations using common (blood, chocolate agar, etc.) growth media (Graham et al., 2007; Willcox, 2013). The ocular surface bacteria mainly include gram-positive, coagulase-negative *Staphylococci* (*S. epidermidis*, *Staphylococcus lentus*, *Staphylococcus xylosus*, and *Staphylococcus sauri*), *Propionibacterium*, *Bacillus*, *Rhodococcus*, *Corynebacterium*, *Klebsiella*, *Erwinia*, *Micrococcus*, *Propionibacterium*, *Streptococcus*, and *Diphtheroid* bacteria (Graham et al., 2007; Miller and Iovieno, 2009). However, bacterial populations on the ocular surface vary significantly with gut microbiota. The density of microbial flora is relatively higher in conjunctiva and eyelids whereas lowest in tears (Willcox, 2013). The bacterial populations that are found in abundance in the gut (e.g., *Bacillus*, *Lactobacillus*, *Enterococcus*, *Escherichia*) are scarce on the ocular surface (e.g., conjunctiva and eyelids) (Miller and Iovieno, 2009). Also, rare population of *Pseudomonas*, *Neisseria*, and *Haemophilus* were also identified in sterile (noninflammatory and noninfectious) conditions on ocular surface. Microbial isolates cultivated from contact lenses also indicated the coagulase-negative *Staphylococcus* as the most frequent genera supporting the aforementioned studies (Willcox, 2013). Another study also reported the abundance of *Staphylococcus coagulase-negative*, *Propionibacterium*, and *Corynebacteria* in the normal human conjunctiva by using three different techniques, i.e., 16S rDNA gene-based deep sequencing, in silico karyotyping, and microbial culturing (120). There are many such studies (Doan et al., 2016; Li et al., 2019b; Miller and Iovieno, 2009; Wen et al., 2017; Willcox, 2013) that reported varying bacterial genera on the healthy ocular surfaces. Wen et al. reported *Staphylococcus*, *Escherichia*, *Propionibacterium*, and *Micrococcus* (Wen et al., 2017) as predominant genera from healthy ocular surfaces. Li et al. identified *Corynebacterium*, *Pseudomonas*, *Chryseobacterium*, *Acinetobacter*, and *Bacillus* as dominant genera in young individuals (Li et al., 2019b), whereas, in elderly individuals, the ocular surface is populated by *Neisseriaceae* or *Corynebacterium* (Suzuki et al., 2020). The ocular surface microbial flora is affected by the host's internal conditions (age, gender, lifestyle, use of contact lens, disease conditions, infection, application of antibiotics, etc.) and simultaneously influenced by external environmental factors. Compared with the ocular surface, the intraocular microbiota is not clearly defined, but it may provide a true reflection of the host ocular microbiome as it is a highly immune privileged site and the chances of external contamination are obscure unless the primary barrier is breached by trauma/injury/infectious conditions (Li et al., 2020). There are various reports demonstrating the association between ocular microbiome dysbiosis and ocular diseases, e.g., dry eye syndrome (tear film defect) and Meibomian gland (located in the eyelid) dysfunction (Li et al., 2019a). However, ocular health is also associated with nonocular host microbiota, i.e., gut microbiota, and various noninfectious inflammatory and autoimmune eye diseases (e.g., AMD (age-related macular degeneration, autoimmune uveitis, glaucoma) in human and animal models reported gut dysbiosis (Heissigerova et al., 2016; Huang et al., 2018; Janowitz et al., 2019; Rosenbaum and Asquith, 2018). Directly targeting the ocular (surface/intraocular) microbiota as therapies against autoimmune and inflammatory ocular diseases is promising; one such study demonstrated that gut microbiome remodeling annihilates the progression of experimental autoimmune uveitis (EAU) by reducing the inflammation (Zhou et al., 2020).

Microbial keratitis (infectious eye disease of the cornea) can cause vision loss and enucleation of eye and is often associated with *P. aeruginosa* (a gram-negative bacteria and it is also

reported to induce keratitis) (Al-Mujaini et al., 2009; Kugadas et al., 2017). *Corynebacterium*, *Sphingomonas*, and *Staphylococcus* are reported to be involved in the meibomian gland dysfunction (Dong et al., 2019; Pinna et al., 2005). However, ocular diseases are not associated with dysbiosis of ocular surfaces, but studies have shown the link between gut−microbiome dysbiosis and ocular diseases such as autoimmune uveitis and Sjögren syndrome (with ocular manifestation) (Kuklinski and Asbell, 2017; Mendez et al., 2020; Moon et al., 2020; de Paiva et al., 2016), again supporting the notion of gut−eye axis cross-talk. *Chlamydia trachomatis* causes trachoma, which is considered one of the leading causes of blindness across the globe, and it is associated with an increase in *Streptococcus* and *Corynebacterium* and reduced bacterial diversity (Zhou et al., 2014b). Ocular neoplasms, e.g., conjunctival papilloma and squamous cell carcinoma are, respectively, associated with human papillomavirus and HIVs (Miller and Iovieno, 2009). The intraocular microenvironment is considered a sterile compartment under steady-state conditions. However, this dogma is challenged by the evidence reporting the translocation of gut microbiota to circulation and other organs due to a breach in the barrier layer under pathological conditions. A similar mechanism is hypothesized for ocular microbiota (Aykut et al., 2019; Kell et al., 2015; Vieira et al., 2018). There are shreds of evidence indicating the presence of microbiota in intraocular compartments, and gut can act as a reservoir for ocular microbiota that may directly or indirectly (via their metabolites, e.g., SCFAs) and often lead to the development of ocular diseases by the mechanism of molecular mimicry (Yadav et al., 2018) (e.g., ocular disease of autoimmune etiology) and dysregulated immunological pathways (e.g., ocular inflammation) (Deng et al., 2021; Nakamura et al., 2016; Smith et al., 2013).

The microbiome-based modulatory approaches as mentioned in the aforementioned sections hold promise in managing ocular diseases when compared to conventional ophthalmic antibiotics as it is accompanied by drawbacks of the ocular microbiome (transient/permanent) dysbiosis, antibiotics resistance, etc., thereby increasing the risk of development of infectious ocular diseases (Miller and Iovieno, 2009). As mentioned before, fecal microbiota transplantation (FMT) that involves isolation of stools from healthy donor and placement of same into the gut of the recipient patient's gut is suffered from limitations such as safety and efficacy over the widely used probiotics (Rosenbaum and Asquith, 2018). Probiotics are the most popular and preferred microbiome-based therapeutic approaches that are thought to mediate various physiological, metabolic, and immunological functions (Shakya et al., 2019; Yadav et al., 2018). Decrease in neutrophil extracellular traps (NETs)−forming potential was observed in presence of *Lactobacilli*; the polysaccharide capsule of *Bacillus fragilis* were protective against autoimmune diseases (Klenkler and Sheardown, 2004; Knop and Knop, 2007). *S. boulardii* and *L. rhamnosus* containing probiotic eye drop formulations (4×10^9 CFU$\times$ ml) were indicated against keratoconjunctivitis (Mangiafico et al., 2012), and *Bifidobacterium* demonstrated its efficacy in reducing the severity of EAU by modulating gut mucosal immunity (via uptake of SCFAs) (Knop and Knop, 2007; Rivière et al., 2016). As mentioned in the previous section, the probiotic formulation containing cocktail of bacterium (i.e., *L. gasseri* KS-13, *Bifidobacterium bifidum* G9-1, and *B. longum* MM-2) improved the symptoms of rhinoconjunctivitis during seasonal allergies (Dennis-Wall et al., 2017). Similarly, dry eye syndrome was ameliorated by the administration of *E. faecium* and *S. boulardii* containing probiotics on tear film (Chisari et al., 2017). Improvement in the clinical manifestation of autoimmune dry eye disease in mouse model was observed after the administration of IRT5

probiotic powder (2×10^8 CFU/gm having five strains of *L. acidophilus, B. bifidum, L. casei, S. thermophilus, and L. reuteri*) (Choi et al., 2020).

Targeted bacteriophage therapy may act as potential alternative for conventional antibiotic therapy particularly in case of antibiotic resistant infectious ocular diseases owing to their high specificity, selectivity, and high replication cycle against the host bacteria (Weinbauer, 2004; Zhang et al., 2021). *P. aeruginosa* causes ulcerative keratitis in dogs, and bacteriophages isolates P2S2 and P5U5 were reported to be an ideal candidate for phage-mediated treatment of disease (Santos et al., 2011). A chimeric phage endolysin, i.e., Ply187AN-KSH3b derived from a prophage (Ply187) has been employed with promising outcome in the treatment of bacterial endophthalmitis (Singh et al., 2014). Phages isolated from sewage water demonstrated specificity against *E. faecalis*, a cytolytic bacterium, and annihilated the alcohol-induced liver injury and hepatosteatosis in gnotobiotic mice (with fecal microbiota transplanted from patients with cytolysin positivity) (Çolakoğlu et al., 2020). The symbiotic gut bacterium *Fusobacterium nucleatum* has been associated with colorectal cancer tumorigenesis by increasing the myeloid-derived suppressor cells (MDSCs). Dong et al. (2020) used bio-inorganic hybrid bacteriophage M13 where capsid was electrostatically coated silver nanoparticles (AgNPs) (M13@Ag) that selectively enhanced the *F. nucleatum* clearance by decreasing the MDSCs. This phage modulatory approach will be instrumental in broad spectrum of infectious ocular diseases and various other human infectious bacterial diseases (Dong et al., 2020). There were many such studies suggesting the immunosuppressive effects of filamentous phages and T4 phage mouse model of EAE and collagen-induced arthritis (Miedzybrodzki et al., 2017; Rakover et al., 2010). Bacteriophages can also be exploited in ocular drug delivery; a subconjunctival injection of stable (chemically and thermodynamically) RNA nanoparticles derived from 3WJ (three-way junction) of pRNA (noncoding promoter-associated RNA) of phi29 (a bacilli bacteriophage) DNA packaging motor revealed increased retention of nanoparticles in the eye (Shi et al., 2018). This phage-mediated strategy may be employed in intraocular drug delivery in the management of ocular diseases. Furthermore, investigation is highly warranted to define the intraocular microbiota through a robust methodology that will aid in developing newer strategies to exploit the gut−ocular microbiota in ocular disease management.

3.4 Skin microbiome

Skin reflects the health of an individual, and there is direct relationship between skin and gut health. Nutrition immensely affects the gut microbiome diversity, and there are growing evidence indicating the intricate relationship along gut−skin axis both inside-out and outside-in. Skin is the largest organ, and skin microbiome composition is largely affected by plethora of factors, e.g., mode of delivery (cesarean vs. normal), age, gender, nutrition, lifestyle, and genetic and environmental factors (humidity, pH). The skin microbiota colonization begins at birth. The bacterial population present on human skin are generally dominated by four genera comprising 52% of *Actinobacteria*, 24% *Firmicutes*, 16% *Proteobacteria*, and 6% *Bacteroidetes* (Grice et al., 2009). Bacterial species that are commonly found on skin from almost all sites of body include *Staphylococcus* sp. (coagulase-negative *S. epidermidis*), *Corynebacterium*, *Cutibacterium acnes*, *Micrococcus*, and *Acinetobacter*, constituting up to 80%

of the entire skin bacterial population (Grice et al., 2009; Samaras, 2020). The commonly found fungi in skin are *Malassezia spp.*, *Aspergillus, Candida albicans, Cryptococcus*, and mites (arthropods) population harboring skin surface/appendages are *Demodex folliculorum* and *Demodex brevis*. Skin virome data of healthy human subjects are scarce, but skin-associated common viruses belonging to families of *Papillomaviridae* (alpha, beta, and gamma human papilloma viruses), *Polyomaviridae, Circoviridae*, and *Coronaviridae* have been reported (Byrd et al., 2018; Kampf et al., 2020). Earlier, it was thought that mother's womb is sterile and human fetus is free from pathogens. But there are emerging studies that revealed the presence of bacteria, archaebacteria, fungi, and even viruses in amniotic and meconium fluid indicating microbial colonization begins prior to the birth (Hansen et al., 2015; Lim et al., 2018; Stinson et al., 2019) however, the associated external contamination cannot be ruled out. During birth, microbiota seeding of fetus skin occurs while passing through mother's birth canal, and microbial communities greatly vary with the mode of delivery. The skin microbiome signature of vaginally delivered baby was found to be dominated by *Lactobacillus spp.* (resembling the signature of vaginal microbiota but reduced in diversity and density), whereas caesarean-delivered baby revealed dominance of *Corynebacterium, Staphylococcus*, and *Cutibacterium* in neonate skin (Scholz and Kilian, 2016) making them susceptible to pathogens and skin-related allergies (Fig. 4.1). Few weeks after birth, the infant skin microbiota become diversified and become populated at various skin specific niches and closely resemble that of mother's skin. However, infant skin and nostrils were found to have unique maternal

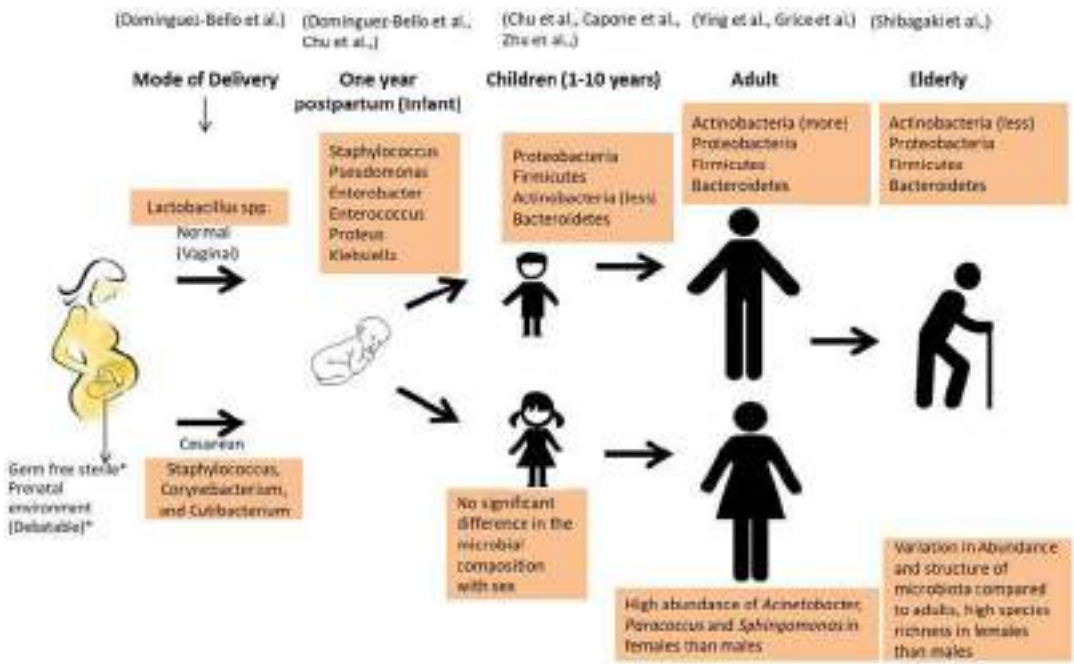

FIGURE 4.1 Schematic representation of preponderance of skin microbiome in prenatal, postnatal (infants), children (1−10 years), and adults (refer to text for description).

signature of *Firmicutes* (e.g., *Staphylococcus*) and *Actinobacteria* (e.g., *Corynebacterium*), and no influence of mode of delivery on skin microbiota diversity observed (Chu et al., 2017). By 1 year of age, infant skin revealed decrease in *Streptococcus* and *Staphylococcus*, and site-specific distribution of microbiota becomes more prominent (Capone et al., 2011). A 12-month follow-up study in infants revealed two main phyla *Firmicutes* and *Proteobacteria* having six predominant bacterial species, i.e., *Staphylococcus, Enterobacter*, *Enterococcus, Proteus, Pseudomonas*, and *Klebsiella* common to mother and infant skin. Zhu et al. revealed that children from 1 to 10 years of age have same microbiota composition as that of adult (i.e., *Actinobacteria, Firmicutes, Proteobacteria,* and *Bacteroidetes*) but with varying ratio along with influence of mode of delivery (Zhu et al., 2019). Furthermore, skin microbiome signature was found to be similar to their mothers supporting previous findings, but no significant gender-based differences were observed. The shift toward puberty brings notable changes in skin microbiome and the adult skin characterized by the dominance of *Actinobacteria spp.* (e.g., lipophilic *Cutibacterium* and *Corynebacterium*), and it may be attributed to excess secretion of sebum in skin (Oh et al., 2012). The physiological differences between males and females may also influence the skin microbial composition due to hormonal variations and sweat/sebum content. Ying et al. observed no significant difference in the basic microbiota community, but the abundance of *Acinetobacter, Paracoccus,* and *Sphingomonas* were found to be higher in females compared with males (Ying et al., 2015). Impact of aging is also a factor that can greatly affect the skin microbiome structure due to various physiological changes, and it may influence with gender, ethnicity, and lifestyle. As mentioned before, gender-based difference observed in adulthood and similar changes, i.e., species abundance (e.g., *Cutibacterium*) was found to be higher in older females than older males (Ying et al., 2015). Shibagaki et al. reported decrease in the richness of *Actinobacteria* (e.g., *Cutibacterium*) with simultaneous increase in *Bacteroidetes, Firmicutes*, and *Proteobacteria* in older individuals compared with adults (Shibagaki et al., 2017). Skins of older women were found to be richer in species of *Actinobacteria (Corynebacterium), Bacteroidetes (Prevotella), Firmicutes (Streptococcus), Proteobacteria (Acinetobacter)* than young/adult women (Shibagaki et al., 2017). Thorough understanding of skin microbiome and skin disease—associated dysbiosis will aid in developing the microbiome-based therapeutic strategies against various skin conditions. Various microbiome-based modulatory approaches in different skin-specific diseases have been described in Table 4.2.

4. Conclusion

Comprehensive understanding of human microbiomes (phylogenetics, metagenomics, and metabolomics), their organ (niche) specific distribution, and interaction with each other (e.g., quorum sensing) or with host is necessary to develop microbiome-based therapeutic strategies. As discussed before, strategies such as probiotics and prebiotics evolved into postbiotics and parabiotics. Fecal microbiome transplantation has been transformed from invasive endoscopic procedures to microencapsulated oral pills. The phage-based therapy has been engineered with introduction of CRISPR/Cas system, and it can be used more precisely against particular microbiota (e.g., engineered bacteriophage against bacterial species). There

TABLE 4.2 Summary of the microbiome-based modulatory approaches employed in skin diseases and the outcome of investigations/trials.

Disease	Study design/Sample number	Intervention	Dose and route of administration	References	Outcome
Psoriasis	Randomized, double-blind, placebo-controlled trial/ N = 90 [probiotics (n = 46) and placebo (n = 44)]	*Bifidobacterium longum CECT 7347, Bacillus lactis CECT 8145 Lactobacillus rhamnosus CECT 8361 mixture*	Oral capsule (1 capsule/day containing 1×10^9CFU probiotic mixture for 12 week)	Navarro-López et al. (2019)	Reduction in PASI score with lower risk of relapse and modulation of microbial composition
Psoriasis	Randomized, double-blind, placebo-controlled interventions/N = 26 [probiotics (n = 12) andand placebo (n = 14)]	*Bifidobacteria infantis (B. infantis) 35,624*	Oral sachets (1 sachet/day containing 1×10^{10} CFU for 8 week)	Groeger et al. (2013)	Reduction in the plasma level of CRP (C-reactive protein) and TNF-α
Refractory metastatic melanoma	Phase I clinical trial/ n = 10 with anti-PD-1-refractory metastatic melanoma	FMT (donors had previously been treated with anti-PD-1 monotherapy for metastatic melanoma and achieved a complete response for at least 1 year)	Colonoscopy and oral stool capsules for 14 days until day 90	Baruch et al. (2021)	Combination of FMT and anti-PD-1 therapy was safe and effective in refractory metastatic melanoma treatment, induced changes in immune cell infiltration
Atopic dermatitis	Open-label phase I/II safety and activity trial (the Beginning Assessment of Cutaneous Treatment Efficacy for Roseomonas in Atopic Dermatitis trial; BACTERiAD I/II)/ N = 15 (10 adults, 5 children)	*Roseomonas mucosa*	Topical spray (1 application, twice per week, for a total of 6 weeks with an initial dose of 10^4 CFU/mL for 2 weeks)	Myles et al. (2018)	Significant decreases in SCORAD and pruritus and *Staphylococcus aureus* microbial burden

Atopic dermatitis	Phase I, double-blind, randomized 1-week trial/N = 54 [probiotics (n = 36); placebo (n = 18)]	*Staphylococcus hominis A9 (ShA9)*	Topical lotion (2g aliquot containing 1×10^6 CFU cm^{-2} for 1 week)	Nakatsuji et al. (2021)	Fewer adverse events with a significant reduction in *S. aureus* colonization and improvement in local inflammation
Atopic dermatitis	Double-blind, randomized, placebo-controlled clinical trial/N = 60 patients [n = 30 probiotics; n = 30 placebo]	Probiatop®	Oral sachet (1g/day containing probiotic mixture of 10^9 CFU each for 6 months)	de Andrade et al. (2022)	Significant reduction in SCORAD
Chronic infected leg ulcers	Prospective uncontrolled study/ N = 34	*Lactobacillus plantarum* (bacteriotherapy)	Topical (culture containing 10^5 CFU/mL once-daily for 10 days)	Peral et al. (2010)	Induced wound healing, reduction in microbial burden, apoptotic and necrotic cells in ulcer beds and increased IL-8 production
Diabetic foot ulcer	Randomized, double-blind, placebo-controlled trial/ N = 60 [probiotics (n = 30); placebo (n = 30)]	*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum,* and *Bifidobacterium bifidum*	Oral capsule (1 capsule/day containing 2×10^9 CFU/g each daily for 12 weeks)	Mohseni et al. (2018)	Reduction in ulcer size and decreased level of total cholesterol, CRP, malondialdehyde and increase in total antioxidant capacity concentrations and nitric oxide
Seborrheic dermatitis	Randomized, double-blind, vehicle-controlled and parallel group comparison study/N = 60	*Vitreoscilla filiformis in La Roche Posay (LRP) spa water*	Topical lotion (5% once daily for 4 weeks)	Guéniche et al. (2008)	Improvement in erythema and scaling score and decrease in pruritus level
Atopic dermatitis (AD)	Observational single-cohort prospective study/n = 320 children	*Produo® Derma*	Oral stick (2 stick/ day containing 1 g synbiotic mixture for a duration of 8 weeks)	Ibáñez et al. (2018)	Reduction in SCORAD and VAS score

(*Continued*)

TABLE 4.2 Summary of the microbiome-based modulatory approaches employed in skin diseases and the outcome of investigations/trials.—cont'd

Disease	Study design/Sample number	Intervention	Dose and route of administration	References	Outcome
Atopic dermatitis	Prospective, controlled, pilot trial/ N = 35 [probiotics (n = 13); placebo (n = 12)]	*Lactobacillus salivarius LS01, Streptococcus thermophilus ST10, and Tara gum*	Oral sachet (1 sachet/day containing 5×10^9 CFU of *L. salivarius*, 2×10^9 CFU of *S. thermophilus* and 125 mg tara gum for 1 month)	Drago et al. (2014)	Improvement in SCORAD score and decrease in microbial burden of *S. aureus*
Atopic dermatitis	Randomized, double-blind, placebo-controlled, parallel-group comparative study/N = 44 [probiotics (n = 22); placebo (n = 22)]	*Bifidobacterium animalis subsp lactis LKM512*	Oral capsules (1 capsule/day containing 6×10^9 CFU for 8 weeks)	Matsumoto et al. (2014)	Reduction in itch score without significant adverse events and increased the expression of kynurenic acid
Atopic dermatitis	Randomized double-blind study/n = 48 [probiotics (n = 32); placebo (n = 16)]	*L. salivarius LS01 Bifidobacterium breve BR03*	Oral sachet (2 sachets/day containing 1×10^9 CFU for 12 weeks)	Iemoli et al. (2012)	Improvement in SCORAD, decreased plasma LPS levels and resulted in immune modulation and tolerance
Atopic dermatitis	Randomized double-blind placebo-controlled study/ n = 38 [probiotics (n = 19); placebo (n = 19)]	*L. salivarius LS01*	Oral sachet (2 sachets/day at a dose of 1×10^9 CFU/g 16 weeks)	Drago et al. (2012)	Improvement in clinical response and modulation of staphylococci load and immune system
Atopic dermatitis	Double-blind, prospective, randomized placebo-controlled study/ n = 220 children [LP (n = 55), LF (n = 53), LP + LF (n = 51); placebo (n = 53)]	*Lactobacillus paracasei (LP) Lactobacillus fermentum (LF)*	Oral capsules (*LP* of 2×10^9 CFU, *LF* of 2×10^9 CFU and *LP* and *LF* mixture of 4×10^9 CFU for 3 months)	Wang et al. (2015)	Probiotic mixture of LP and LF resulted in lower SCORAD score and improving quality of life

are emerging evidence that paved the way for microbiome-based therapeutics as an attractive modern medicine in near future, and it has been extensively explored in almost all fields of medicine, some of which has already been discussed in this chapter. In future, microbiomes and their metabolites-based therapeutics will be encompassing broad spectrum of human diseases and can also be exploited in diagnostics.

References

Ahmed, V., Kumar, J., Kumar, M., Chauhan, M.B., Vij, M., Ganguli, M., Chauhan, N.S., 2013. Synthesis, characterization of penicillin G capped silver nanoconjugates to combat β-Lactamase resistance in infectious microorganism. J. Biotechnol. 163, 419–424. https://doi.org/10.1016/j.jbiotec.2012.12.002.

Ahmed, V., Verma, M.K., Gupta, S., Mandhan, V., Chauhan, N.S., 2018. Metagenomic profiling of soil microbes to mine salt stress tolerance genes. Front. Microbiol. 9, 159. https://doi.org/10.3389/fmicb.2018.00159.

Al-Mujaini, A., Al-Kharusi, N., Thakral, A., Wali, U.K., 2009. Bacterial keratitis: perspective on epidemiology, clinicopathogenesis, diagnosis and treatment. Sultan Qaboos Univ. Med. J. 9 (2), 184–195.

Aleti, G., Baker, J.L., Tang, X., Alvarez, R., Dinis, M., Tran, N.C., et al., 2019. Identification of the bacterial biosynthetic gene clusters of the oral microbiome illuminates the unexplored social language of bacteria during health and disease. mBio 10 (2), 00321-19.

Allen-Vercoe, E., Reid, G., Viner, N., Gloor, G.B., Hota, S., Kim, P., et al., 2012. A Canadian Working Group report on fecal microbial therapy: microbial ecosystems therapeutics. Can. J. Gastroenterol. 26 (7), 457–462.

Arweiler, N.B., Auschill, T.M., Heumann, C., Hellwig, E., Al-Ahmad, A., 2020. Influence of probiotics on the salivary microflora oral Streptococci and their integration into oral biofilm. Antibiotics 9 (11).

Aykut, B., Pushalkar, S., Chen, R., Li, Q., Abengozar, R., Kim, J.I., et al., 2019. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. Nature 574 (7777), 264–267.

Baquero, F., Nombela, C., 2012. The microbiome as a human organ. Clin. Microbiol. Infect. 4, 2–4.

Baruch, E.N., Youngster, I., Ben-Betzalel, G., Ortenberg, R., Lahat, A., Katz, L., et al., 2021. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 371 (6529), 602–609.

Bassis, C.M., Tang, A.L., Young, V.B., Pynnonen, M.A., 2014. The nasal cavity microbiota of healthy adults. Microbiome 2 (27), 2049–2618.

Bäuerl, C., Abitayeva, G., Sosa-Carrillo, S., Mencher-Beltrán, A., Navarro-Lleó, N., Coll-Marqués, J.M., et al., 2019. P40 and P75 are singular functional muramidases present in the Lactobacillus casei/paracasei/rhamnosus taxon. Front. Microbiol. 10 (1420).

Belkaid, Y., Hand, T.W., 2014. Role of the microbiota in immunity and inflammation. Cell 157 (1), 121–141.

Berg, R.D., 1996. The indigenous gastrointestinal microflora. Trends Microbiol. 4 (11), 430–435.

Biesbroek, G., Bosch, A.A., Wang, X., Keijser, B.J., Veenhoven, R.H., Sanders, E.A., et al., 2014. The impact of breastfeeding on nasopharyngeal microbial communities in infants. Am. J. Respir. Crit. Care Med. 190 (3), 298–308.

Biesbroek, G., Tsivtsivadze, E., Sanders, E.A., Montijn, R., Veenhoven, R.H., Keijser, B.J., et al., 2014. Early respiratory microbiota composition determines bacterial succession patterns and respiratory health in children. Am. J. Respir. Crit. Care Med. 190 (11), 1283–1292.

Borody, T.J., G, L., Andrews, P., Brandl, S., Noonan, S., Cole, P., Hyland, L., Morgan, A., Maysey, J., Moore-Jones, D., 1989. Bowel-flora alteration: a potential cure for inflammatory bowel disease and irritable bowel syndrome? Med J Aust. [Case Reports] 150 (10), 604.

Byrd, A.L., Belkaid, Y., Segre, J.A., 2018. The human skin microbiome. Nat. Rev. Microbiol. 16 (3), 143–155.

Calderón-Oliver, M., P-A, E., 2022. The role of microencapsulation in food application. Molecules 27 (5), 1499.

Cani, P.D., Dewever, C., Delzenne, N.M., 2004. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br. J. Nutr. 92 (3), 521–526.

Capone, K.A., Dowd, S.E., Stamatas, G.N., Nikolovski, J., 2011. Diversity of the human skin microbiome early in life. J. Invest. Dermatol. 131 (10), 2026–2032.

Caselli, E., Fabbri, C., D'Accolti, M., et al., 2020. Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. BMC Microbiol. 20 (1), 120.

Center for Biologics Evaluation and Research (FDA-2013-D-0811), 2016. In: Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat *Clostridium difficile* Infection Not Responsive to Standard Therapies. U.S. Food and Drug Administration.

Centurion, F., Basit, A.W., Liu, J., Gaisford, S., Rahim, M.A., Kalantar-Zadeh, K., 2021. Nanoencapsulation for probiotic delivery. ACS Nano 15 (12), 18653–18660.

Chauhan, N.S., Nain, S., Sharma, R., 2017. Identification of arsenic resistance genes from marine sediment metagenome. Indian J. Microbiol. 299–306. https://doi.org/10.1007/s12088-017-0658-0.

Chauhan, N.S., Pandey, R., Mondal, A.K., Gupta, S., Verma, M.K., Jain, S., Ahmed, V., Patil, R., Agarwal, D., Girase, B., Shrivastava, A., Mobeen, F., Sharma, V., Srivastava, T.P., Juvekar, S.K., Prasher, B., Mukerji, M., Dash, D., 2018. Western Indian rural gut microbial diversity in extreme *prakriti* endo-phenotypes reveals signature microbes. Front. Microbiol. 9, 118. https://doi.org/10.3389/fmicb.2018.00118.

Chauhan, N.S., 2019. Metagenome analysis and interpretation. In: Misra, G. (Ed.), Data Processing Handbook for Complex Biological Data Sources. Academic press, USA, pp. 139–160. https://doi.org/10.1016/B978-0-12-816548-5.00010-1.

Chen, L., Jia, L., Zhang, Q., Zhou, X., Liu, Z., Li, B., et al., 2017. A novel antimicrobial peptide against dental-caries-associated bacteria. Anaerobe 47, 165–172.

Chisari, G., Chisari, E.M., Borzi, A.M., Chisari, C.G., 2017. Aging eye microbiota in dry eye syndrome in patients treated with Enterococcus faecium and Saccharomyces boulardii. Curr. Clin. Pharmacol. 12 (2), 99–105.

Choi, S.H., Oh, J.W., Ryu, J.S., Kim, H.M., Im, S.H., Kim, K.P., et al., 2020. IRT5 probiotics changes immune modulatory protein expression in the extraorbital lacrimal glands of an autoimmune dry eye mouse model. Invest. Ophthalmol. Vis. Sci. 61 (3), 42.

Chu, D.M., Ma, J., Prince, A.L., Antony, K.M., Seferovic, M.D., Aagaard, K.M., 2017. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat. Med. 23 (3), 314–326.

Clarke, G., Stilling, R.M., Kennedy, P.J., Stanton, C., Cryan, J.F., Dinan, T.G., 2014. Minireview: gut microbiota: the neglected endocrine organ. Mol. Endocrinol. 28 (8), 1221–1238.

Çolakoğlu, M., Xue, J., Trajkovski, M., 2020. Bacteriophage prevents alcoholic liver disease. Cell 180 (2), 218–220.

Dassi, E., Ferretti, P., Covello, G., Speccher, A., Migazzi, A., Bosco, B., et al., 2018. The short-term impact of probiotic consumption on the oral cavity microbiome. Sci. Rep. 8 (1), 10476.

de Andrade, P.D.S.M.A., Maria E Silva, J., Carregaro, V., Sacramento, L.A., Roberti, L.R., Aragon, D.C., et al., 2022. Efficacy of probiotics in children and adolescents with atopic dermatitis: a randomized, double-blind, placebo-controlled study. Front. Nutr. 8, 833666.

de Paiva, C.S., Jones, D.B., Stern, M.E., Bian, F., Moore, Q.L., Corbiere, S., et al., 2016. Altered mucosal microbiome diversity and disease severity in sjögren syndrome. Sci. Rep. 6, 23561.

Deng, Y., Ge, X., Li, Y., Zou, B., Wen, X., Chen, W., et al., 2021. Identification of an intraocular microbiota. Cell Discov. 7 (1), 13, 2021/03/09.

Dennis-Wall, J.C., Culpepper, T., Nieves Jr., C., Rowe, C.C., Burns, A.M., Rusch, C.T., et al., 2017. Probiotics (Lactobacillus gasseri KS-13, Bifidobacterium bifidum G9-1, and Bifidobacterium longum MM-2) improve rhinoconjunctivitis-specific quality of life in individuals with seasonal allergies: a double-blind, placebo-controlled, randomized trial. Am. J. Clin. Nutr. 105 (3), 758–767.

Dewhirst, F.E., Chen, T., Izard, J., Paster, B.J., Tanner, A.C., Yu, W.H., et al., 2010. The human oral microbiome. J. Bacteriol. 192 (19), 5002–5017.

Dhakan, D.B., Maji, A., Sharma, A.K., Saxena, R., Pulikkan, J., Grace, T., et al., 2019. The unique composition of Indian gut microbiome, gene catalogue, and associated fecal metabolome deciphered using multi-omics approaches. Gigascience 8 (3), giz004.

Di Cerbo, A., Palmieri, B., Aponte, M., Morales-Medina, J.C., Iannitti, T., 2016. Mechanisms and therapeutic effectiveness of lactobacilli. J. Clin. Pathol. 69 (3), 187–203.

Doan, T., Akileswaran, L., Andersen, D., Johnson, B., Ko, N., Shrestha, A., et al., 2016. Paucibacterial microbiome and resident DNA virome of the healthy conjunctiva. Invest. Ophthalmol. Vis. Sci. 57 (13), 5116–5126.

Dominguez-Bello, M.G., Costello, E.K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., et al., 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc. Natl. Acad. Sci. U S A 107 (26), 11971–11975.

Dong, X., Wang, Y., Wang, W., Lin, P., Huang, Y., 2019. Composition and diversity of bacterial community on the ocular surface of patients with meibomian gland dysfunction. Invest. Ophthalmol. Vis. Sci. 60 (14), 4774–4783.

Dong, X., Pan, P., Zheng, D., Bao, P., Zeng, X., Zhang, X.Z., 2020. Bioinorganic hybrid bacteriophage for modulation of intestinal microbiota to remodel tumor-immune microenvironment against colorectal cancer. Sci. Adv. 6.

Donia, M.S., Cimermancic, P., Schulze, C.J., Wieland Brown, L.C., Martin, J., Mitreva, M., et al., 2014. A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. Cell 158 (6), 1402–1414.

Drago, L., Toscano, M., De Vecchi, E., Piconi, S., Iemoli, E., 2012. Changing of fecal flora and clinical effect of L. salivarius LS01 in adults with atopic dermatitis. J. Clin. Gastroenterol. 46 (Suppl. l), S56–S63.

Drago, L., De Vecchi, E., Toscano, M., Vassena, C., Altomare, G., Pigatto, P., 2014. Treatment of atopic dermatitis eczema with a high concentration of Lactobacillus salivarius LS01 associated with an innovative gelling complex: a pilot study on adults. J. Clin. Gastroenterol. 48 (Suppl. 1), S47–S51.

Duan, F.F., Liu, J.H., March, J.C., 2015. Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes. Diabetes 64 (5), 1794–1803.

Duan, Y., Llorente, C., Lang, S., Brandl, K., Chu, H., Jiang, L., et al., 2019. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature 575 (7783), 505–511.

Durrer, K.E., Allen, M.S., Hunt von Herbing, I., 2017. Genetically engineered probiotic for the treatment of phenylketonuria (PKU); assessment of a novel treatment in vitro and in the PAHenu2 mouse model of PKU. PLoS ONE 12 (5), e0176286.

Eckert, R., He, J., Yarbrough, D.K., Qi, F., Anderson, M.H., Shi, W., 2006. Targeted killing of Streptococcus mutans by a pheromone-guided "smart" antimicrobial peptide. Antimicrob. Agents. Chemother. 50 (11), 3651–3657.

Eiseman, B., Silen, W., Bascom, G.S., Kauvar, A.J., 1958. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery 44 (5), 854–859.

Endam, L.M., Alromaih, S., Gonzalez, E., Madrenas, J., Cousineau, B., Renteria, A.E., et al., 2020. Intranasal application of Lactococcus lactis W136 is safe in chronic rhinosinusitis patients with previous sinus surgery. Front. Cell Infect. Microbiol. 10, 440.

Feuerstadt, P., Louie, T.J., Lashner, B., Wang, E.E.L., Diao, L., Bryant, J.A., et al., 2022. SER-109, an oral microbiome therapy for recurrent Clostridioides difficile infection. N. Engl. J. Med. 386 (3), 220–229.

Fung, T.C., Bessman, N.J., Hepworth, M.R., Kumar, N., Shibata, N., Kobuley, D., et al., 2016. Lymphoid-tissue-resident commensal bacteria promote members of the IL-10 cytokine family to establish mutualism. Immunity 44 (3), 634–646.

Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., Mulder, I., Lan, A., Bridonneau, C., et al., 2009. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 31 (4), 677–689.

Galdeano, C.M., Perdigón, G., 2006. The probiotic bacterium Lactobacillus casei induces activation of the gut mucosal immune system through innate immunity. Clin. Vacc. Immunol. 13 (2), 219–226.

Garcia, S.S., Blackledge, M.S., Michalek, S., Su, L., Ptacek, T., Eipers, P., et al., 2017. Targeting of Streptococcus mutans biofilms by a novel small molecule prevents dental caries and preserves the oral microbiome. J Dent Res 96 (7), 807–814.

Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., et al., 2017. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat. Rev. Gastroenterol. Hepatol. 14 (8), 491–502.

Graham, J.E., Moore, J.E., Jiru, X., Moore, J.E., Goodall, E.A., Dooley, J.S.G., et al., 2007. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. Invest. Ophthalmo. Vis. Sci. 48 12, 5616–5623.

Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C., et al., 2009. Topographical and temporal diversity of the human skin microbiome. Science (New York, NY) 324 (5931), 1190–1192.

Groeger, D., O'Mahony, L., Murphy, E.F., Bourke, J.F., Dinan, T.G., Kiely, B., et al., 2013. Bifidobacterium infantis 35624 modulates host inflammatory processes beyond the gut. Gut Microbes 4 (4), 325–339.

Guéniche, A., Cathelineau, A.C., Bastien, P., Esdaile, J., Martin, R., Queille Roussel, C., et al., 2008. Vitreoscilla filiformis biomass improves seborrheic dermatitis. J. Eur. Acad. Dermatol. Venereol. 22 (8), 1014–1015.

Guo, L., McLean, J.S., Yang, Y., Eckert, R., Kaplan, C.W., Kyme, P., et al., 2015. Precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology. Proc. Natl. Acad. Sci. 112 (24), 7569–7574.

Gupta, S., Kumar, M., Kumar, J., et al., 2017. Systemic analysis of soil microbiome deciphers anthropogenic influence on soil ecology and ecosystem functioning. Int. J. Environ. Sci. Technol. 14, 2229–2238. https://doi.org/10.1007/s13762-017-1301-7.

Gupta, S., Shariff, M., Chaturvedi, G., Sharma, A., Goel, N., Yadav, M., Mortensen, M.S., Sørensen, S.J., Mukerji, M., Chauhan, N.S., 2021. Comparative Analysis of the alveolar microbiome in COPD, ECOPD, Sarcoidosis, and ILD patients to identify respiratory illnesses specific microbial signatures. Sci. Rep. 11, 3963. https://doi.org/10.1038/s41598-021-83524-2.

Guslandi, M., M, G., Sorghi, M., Testoni, P.A., 2000. Saccharomyces boulardii in maintenance treatment of Crohn's disease. Dig. Dis. Sci. [Clinical Trial] 45 (7), 1462–1464.

Guslandi, M., G, P., Alberto Testoni, P., 2003. A pilot trial of Saccharomyces boulardii in ulcerative colitis. Eur. J. Gastroenterol. Hepatol. [Clinical Trial] 15 (6), 697–698.

Habermann, W., Zimmermann, K., Skarabis, H., Kunze, R., Rusch, V., 2002. [Reduction of acute recurrence in patients with chronic recurrent hypertrophic sinusitis by treatment with a bacterial immunostimulant (*Enterococcus faecalis* Bacteriae of human origin]. Arzneimittelforschung 52 (8), 622–627.

Hansen, R., Scott, K.P., Khan, S., Martin, J.C., Berry, S.H., Stevenson, M., et al., 2015. First-pass meconium samples from healthy term vaginally-delivered neonates: an analysis of the microbiota. PLoS ONE 10 (7), e0133320–e.

Heissigerova, J., Seidler Stangova, P., Klimova, A., Svozilkova, P., Hrncir, T., Stepankova, R., et al., 2016. The microbiota determines susceptibility to experimental autoimmune uveoretinitis. J. Immuno. Res. 2016, 5065703.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., et al., 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11 (8), 506–514.

Hillman, J.D., Mo, J., McDonell, E., Cvitkovitch, D., Hillman, C.H., 2007. Modification of an effector strain for replacement therapy of dental caries to enable clinical safety trials. J. Appl. Microbiol. 102 (5), 1209–1219.

Hogan, D.A., Kolter, R., 2002. Pseudomonas-Candida interactions: an ecological role for virulence factors. Science 296 (5576), 2229–2232.

Htwe, M.M., Teanpaisan, R., Khongkow, P., Amnuaikit, T., 2019. Liposomes of probiotic's lyophilized cell free supernatant; a potential cosmeceutical product. Pharmazie 74 (8), 462–466.

Huang, X., Palmer, S.R., Ahn, S.J., Richards, V.P., Williams, M.L., Nascimento, M.M., et al., 2016. A highly arginolytic Streptococcus species that potently antagonizes Streptococcus mutans. Appl. Environ. Microbiol. 82 (7), 2187–2201.

Huang, X., Ye, Z., Cao, Q., Su, G., Wang, Q., Deng, J., et al., 2018. Gut microbiota composition and fecal metabolic phenotype in patients with acute anterior uveitis. Invest. Ophthalmol. Vis. Sci. 59 (3), 1523–1531.

Ibáñez, M.D., Rodríguez Del Río, P., González-Segura Alsina, D., Villegas Iglesias, V., 2018. Effect of synbiotic supplementation on children with atopic dermatitis: an observational prospective study. Eur. J. Pediatr. 177 (12), 1851–1858.

Iemoli, E., Trabattoni, D., Parisotto, S., Borgonovo, L., Toscano, M., Rizzardini, G., et al., 2012. Probiotics reduce gut microbial translocation and improve adult atopic dermatitis. J. Clin. Gastroenterol. 46, S33–S40.

Ishida, Y., Nakamura, F., Kanzato, H., Sawada, D., Hirata, H., Nishimura, A., et al., 2005. Clinical effects of Lactobacillus acidophilus strain L-92 on perennial allergic rhinitis: a double-blind, placebo-controlled study. J. Dairy Sci. 88 (2), 527–533.

Janowitz, C., Nakamura, Y.K., Metea, C., Gligor, A., Yu, W., Karstens, L., et al., 2019. Disruption of intestinal homeostasis and intestinal microbiota during experimental autoimmune uveitis. Invest. Ophthalmol. Vis. Sci. 60 (1), 420–429.

Jerzynska, J., Stelmach, W., Balcerak, J., Woicka-Kolejwa, K., Rychlik, B., Blauz, A., et al., 2016. Effect of Lactobacillus rhamnosus GG and vitamin D supplementation on the immunologic effectiveness of grass-specific sublingual immunotherapy in children with allergy. Allergy Asthma Proc. 37 (4), 324–334.

Jørn, A.A., P, B.J., Stokes, L.N., Olsen, I., Dewhirst, F.E., 2005. Defining the normal bacterial flora of the oral cavity. J. Clin. Microbiol. 43 (11), 5721–5732.

Kampf, G., Todt, D., Pfaender, S., Steinmann, E., March 01, 2020. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J. Hosp. Infec. [Review] 104 (3), 236–251.

Kapil, V., Webb, A.J., Ahluwalia, A., 2010. Inorganic nitrate and the cardiovascular system. Heart 96 (21), 1703–1709.

Kell, D., Potgieter, M., Pretorius, E., 2015. Individuality, phenotypic differentiation, dormancy and 'persistence' in culturable bacterial systems: commonalities shared by environmental, laboratory, and clinical microbiology. F1000Res 1000 (179), 4.

Klenkler, B., Sheardown, H., 2004. Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. Exp. Eye Res. 79 (5), 677–688.

Knop, E., Knop, N., 2007. Anatomy and immunology of the ocular surface. Chem. Immunol. Allergy 92, 36–49.

Kugadas, A., Wright, Q., Geddes-McAlister, J., Gadjeva, M., 2017. Role of microbiota in strengthening ocular mucosal barrier function through secretory IgA. Invest. Ophthalmol. Vis. Sci. 58 (11), 4593–4600.

Kuklinski, E., Asbell, P.A., 2017. Sjogren's syndrome from the perspective of ophthalmology. Clin. Immunol. 182, 55–61.

Kumar, J., Kumar, M., Gupta, S., Ahmed, V., Bhambi, M., Pandey, R., Chauhan, N.S., 2016. An improved methodology to overcome key issues associated with the methods of human fecal metagenomic DNA extraction. Genom. Proteom. Bioinform. 14, 371–378. https://doi.org/10.1016/j.gpb.2016.06.002.

Kumar, J., Kumar, M., Pandey, R., Chauhan, N.S., 2017. Physiopathology and management of gluten-induced celiac disease. J. Food Sci. 82, 270–277. https://doi.org/10.1111/1750-3841.13612.

Kumar, J., Verma, M.K., Kumar, T., Pandey, R., Yadav, M., Chauhan, N.S., 2018. S9A serine protease engender antigenic gluten catabolic competence to the human gut microbe. Indian. J. Microbiol. 58, 294–300. https://doi.org/10.1007/s12088-018-0732-2.

Kumar, T., Pandey, R., Chauhan, N.S., 2020. Hypoxia inducible factor-1α: the curator of gut homeostasis. Front. Cell. Infect. Microbiol. 10, 227. https://doi.org/10.3389/fcimb.2020.00227.

Kumar, N., Mittal, A., Yadav, M., Sharma, S., Kumar, T., Chakraborty, R., Sengupta, S., Chauhan, N.S., 2021. Photocatalytic TiO2/CdS/ZnS nanocomposite induces *Bacillus subtilis* cell death by disrupting its metabolism and membrane integrity. Indian J. Microbiol. 61, 487–496. https://doi.org/10.1007/s12088-021-00973-z.

Kurtz, C.B., Millet, Y.A., Puurunen, M.K., Perreault, M., Charbonneau, M.R., Isabella, V.M., et al., 2019. An engineered *E. coli* Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. Sci. Transl. Med. 11 (475), eaau7975.

Lederberg, J., McCray, A.T., 2001. `Ome sweet `Omics–A genealogical treasury of words. Scientist 15, 8.

Lemon, K.P., Klepac-Ceraj, V., Schiffer, H.K., Brodie, E.L., Lynch, S.V., Kolter, R., 2010. Comparative analyses of the bacterial microbiota of the human nostril and oropharynx. mBio 1 (3) e00129-10.

Li, Z., Gong, Y., Chen, S., Li, S., Zhang, Y., Zhong, H., et al., 2019a. Comparative portrayal of ocular surface microbe with and without dry eye. J. Microbiol. 57 (11), 1025–1032.

Li, C., Bei, T., Niu, Z., Guo, X., Wang, M., Lu, H., et al., 2019b. Adhesion and colonization of the probiotic Lactobacillus rhamnosus labeled by Dsred2 in mouse gut. Curr. Microbiol. 76 (7), 896–903.

Li, J.J., Yi, S., Wei, L., 2020. Ocular microbiota and intraocular inflammation. Front. Immuno. 11, 609765.

Lim, E.S., Rodriguez, C., Holtz, L.R., 2018. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. Microbiome 6 (1), 018–0475.

Lin, W.Y., Fu, L.S., Lin, H.K., Shen, C.Y., Chen, Y.J., 2014. Evaluation of the effect of Lactobacillus paracasei (HF.A00232) in children (6-13 years old) with perennial allergic rhinitis: a 12-week, double-blind, randomized, placebo-controlled study. Pediatr. Neonatol. 55 (3), 181–188.

Liu, Y.L., Nascimento, M., Burne, R.A., 2012. Progress toward understanding the contribution of alkali generation in dental biofilms to inhibition of dental caries. Int. J. Oral Sci. 4 (3), 135–140.

López-López, A., Camelo-Castillo, A., Ferrer, M.D., Simon-Soro, Á., Mira, A., 2017. Health-associated niche inhabitants as oral probiotics: the case of Streptococcus dentisani. Front. Microbiol. 8, 379.

Luna, P.N., Hasegawa, K., Ajami, N.J., Espinola, J.A., Henke, D.M., Petrosino, J.F., et al., 2018. The association between anterior nares and nasopharyngeal microbiota in infants hospitalized for bronchiolitis. Microbiome 6 (1), 2.

Lundberg, J.O., Gladwin, M.T., Ahluwalia, A., Benjamin, N., Bryan, N.S., Butler, A., et al., 2009. Nitrate and nitrite in biology, nutrition and therapeutics. Nat. Chem. Biol. 5 (12), 865–869.

Lundberg, J.O., Carlström, M., Larsen, F.J., Weitzberg, E., 2011. Roles of dietary inorganic nitrate in cardiovascular health and disease. Cardiovasc. Res. 89 (3), 525–532.

Luo, J., Wang, T., Liang, S., Hu, X., Li, W., Jin, F., 2014. Ingestion of Lactobacillus strain reduces anxiety and improves cognitive function in the hyperammonemia rat. Sci. China Life Sci. 57 (3), 327–335.

Machiulskiene, V., Nyvad, B., Baelum, V., 2001. Caries preventive effect of sugar-substituted chewing gum. Commun. Dent. Oral Epidemiol. 29 (4), 278–288.

Maini Rekdal, V., Bess, E.N., Bisanz, J.E., Turnbaugh, P.J., Balskus, E.P., 2019. Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. Science 364 (6445), eaau6323.

Mangiafico, S., Aleo, D., Saita, M.G., Cro, M.G., Mangiafico, S., 2012. A stable aqueous formulation of probiotics for topical ocular therapy. Invest. Ophthalmol. Vis. Sci. 53 (14), 2757.

Mårtensson, A., Abolhalaj, M., Lindstedt, M., Mårtensson, A., Olofsson, T.C., Vásquez, A., et al., 2017. Clinical efficacy of a topical lactic acid bacterial microbiome in chronic rhinosinusitis: a randomized controlled trial. Laryngoscope Investig. Otolaryngol. 2 (6), 410–416.

Martin, A.M., Sun, E.W., Rogers, G.B., Keating, D.J., 2019. The influence of the gut microbiome on host metabolism through the regulation of gut hormone release. Front. Physiol. 10 (428).

Matsumoto, M., Ebata, T., Hirooka, J., Hosoya, R., Inoue, N., Itami, S., et al., 2014. Antipruritic effects of the probiotic strain LKM512 in adults with atopic dermatitis. Ann. Allergy Asthma Immunol. 113 (2), 209–216.

Mehta, P., Yadav, M., Ahmed, V., Goyal, K., Pandey, R., Chauhan, N.S., 2021. Culture-independent exploration of the hypersaline ecosystem indicates the environment-specific microbiome evolution. Front. Microbiol. 12, 686549. https://doi.org/10.3389/fmicb.2021.686549.

Mendez, R., Watane, A., Farhangi, M., Cavuoto, K.M., Leith, T., Budree, S., et al., 2020. Gut microbial dysbiosis in individuals with Sjögren's syndrome. Microbial. Cell Factor. 19 (1), 90.

Miedzybrodzki, R., Borysowski, J., Kłak, M., Jończyk-Matysiak, E., Obmińska-Mrukowicz, B., Suszko-Pawłowska, A., et al., 2017. In vivo studies on the influence of bacteriophage preparations on the autoimmune inflammatory process. Biomed. Res. Int. 3612015 (10), 22.

Mika, M., Mack, I., Korten, I., Qi, W., Aebi, S., Frey, U., et al., 2015. Dynamics of the nasal microbiota in infancy: a prospective cohort study. J. Allergy Clin. Immunol. 135 (4), 905–912.

Miller, D., Iovieno, A., 2009. The role of microbial flora on the ocular surface. Curr. Opin. Allergy Clin. Immunol. 9 (5), 466–470.

Mittal, A., Kumar, N., Chauhan, N.S., 2019. Curcumin encapsulated PEGylated nanoliposomes: a potential anti-infective therapeutic agent. Indian J. Microbiol59 336–343. https://doi.org/10.1007/s12088-019-00811-3.

Mohseni, S., Bayani, M., Bahmani, F., Tajabadi-Ebrahimi, M., Bayani, M.A., Jafari, P., et al., 2018. The beneficial effects of probiotic administration on wound healing and metabolic status in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. Diabet. Metab. Res. Rev. 34 (3), 21.

Mondal, A.K., Kumar, J., Pandey, R., Gupta, S., Kumar, M., Bansal, G., Mukerji, M., Dash, D., Chauhan, N.S., 2017. Comparative genomics of host–symbiont and free-living *oceanobacillus* species. Genom. Biol. Evol. 9, 1175–1182. https://doi.org/10.1093/gbe/evx076.

Monika, Verma, M.K., Ahmed, V., Chauhan, N.S., 2017. Human gut microbiome: an imperative element for human survival. Curr. Trends Biomed. Eng. Biosci. 6 (1). https://doi.org/10.19080/CTBEB.2017.06.555680 (IF:1.126).

Moon, J., Choi, S.H., Yoon, C.H., Kim, M.K., 2020. Gut dysbiosis is prevailing in Sjögren's syndrome and is related to dry eye severity. PLoS ONE 15 (2), e0229029–e.

Mukerji, S.S., Pynnonen, M.A., Kim, H.M., Singer, A., Tabor, M., Terrell, J.E., 2009. Probiotics as adjunctive treatment for chronic rhinosinusitis: a randomized controlled trial. Otolaryngol. Head Neck Surg. 140 (2), 202–208.

Myles, I.A., Earland, N.J., Anderson, E.D., Moore, I.N., Kieh, M.D., Williams, K.W., et al., 2018. First-in-human topical microbiome transplantation with Roseomonas mucosa for atopic dermatitis. JCI Insight 3 (9), 120608.

Nakamura, Y.K., Metea, C., Karstens, L., Asquith, M., Gruner, H., Moscibrocki, C., et al., 2016. Gut microbial alterations associated with protection from autoimmune uveitis. Invest. Ophthalmol. Vis. Sci. 57 (8), 3747–3758.

Nakatsuji, T., Hata, T.R., Tong, Y., Cheng, J.Y., Shafiq, F., Butcher, A.M., et al., 2021. Development of a human skin commensal microbe for bacteriotherapy of atopic dermatitis and use in a phase 1 randomized clinical trial. Nat. Med. 27 (4), 700–709.

Nascimento, M.M., 2018. Potential uses of arginine in dentistry. Adv. Dent. Res. 29 (1), 98–103.

Navarro-López, V., Martínez-Andrés, A., Ramírez-Boscá, A., Ruzafa-Costas, B., Núñez-Delegido, E., Carrión-Gutiérrez, M.A., et al., 2019. Efficacy and safety of oral administration of a mixture of probiotic strains in patients with psoriasis: a randomized controlled clinical trial. Acta Derm. Venereol. 99 (12), 1078–1084.

Nielsen, D.S., Cho, G.S., Hanak, A., Huch, M., Franz, C.M., Arneborg, N., 2010. The effect of bacteriocin-producing Lactobacillus plantarum strains on the intracellular pH of sessile and planktonic Listeria monocytogenes single cells. Int. J. Food Microbiol. 31 (141), 14.

Nishiyama, K., Seto, Y., Yoshioka, K., Kakuda, T., Takai, S., Yamamoto, Y., et al., 2014. Lactobacillus gasseri SBT2055 reduces infection by and colonization of Campylobacter jejuni. PLoS ONE 9 (9), e108827.

Oh, J., Conlan, S., Polley, E.C., Segre, J.A., Kong, H.H., 2012. Shifts in human skin and nares microbiota of healthy children and adults. Genom. Med. 4 (10), 77, 2012/10/10.

Ott, S.J., Waetzig, G.H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J.A., et al., 2017. Efficacy of sterile fecal filtrate transfer for treating patients with *Clostridium difficile* infection. Gastroenterology 152 (4), 799–811.

Peral, M.C., Rachid, M.M., Gobbato, N.M., Huaman Martinez, M.A., Valdez, J.C., 2010. Interleukin-8 production by polymorphonuclear leukocytes from patients with chronic infected leg ulcers treated with Lactobacillus plantarum. Clin. Microbiol. Infect. 16 (3), 281–286.

Perez, R.H., Zendo, T., Sonomoto, K., 2014. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microbial. Cell Factor. 13 (Suppl. 1), S3-S. Suppl 1.

Petersen, P.E., Razanamihaja, N., 1999. Carbamide-containing polyol chewing gum and prevention of dental caries in schoolchildren in Madagascar. Int. Dent. J. 49 (4), 226–230.

Pflughoeft, K.J., Versalovic, J., 2012. Human microbiome in health and disease. Annu. Rev. Pathol. 7, 99–122.

Phukan, N., Brooks, A.E.S., Simoes-Barbosa, A., 2018. A cell surface aggregation-promoting factor from Lactobacillus gasseri contributes to inhibition of Trichomonas vaginalis adhesion to human vaginal ectocervical cells. Infect. Immun. 86 (8), 00907–00917.

Pinna, A., Sechi, L.A., Zanetti, S., Carta, F., 2005. Detection of virulence factors in a corneal isolate of *Klebsiella pneumoniae*. Ophthalmology 112 (5), 883–887.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., et al., 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464 (7285), 59–65.

Rakover, I.S., Zabavnik, N., Kopel, R., Paz-Rozner, M., Solomon, B., 2010. Antigen-specific therapy of EAE via intranasal delivery of filamentous phage displaying a myelin immunodominant epitope. J. Neuroimmunol. 225 (1–2), 68–76.

Ramakrishnan, V.R., Feazel, L.M., Gitomer, S.A., Ir, D., Robertson, C.E., Frank, D.N., 2013. The microbiome of the middle meatus in healthy adults. PLoS ONE 8 (12), e85507-e.

Rasmussen, T.S., Mentzel, C.M.J., Kot, W., Castro-Mejía, J.L., Zuffa, S., Swann, J.R., et al., 2020. Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model. Gut 69 (12), 2122–2130.

Raveh-Sadka, T., Thomas, B.C., Singh, A., Firek, B., Brooks, B., Castelle, C.J., et al., 2015. Gut bacteria are rarely shared by co-hospitalized premature infants, regardless of necrotizing enterocolitis development. Elife 3 (4), e05477.

Rivière, A., Selak, M., Lantin, D., Leroy, F., De Vuyst, L., 2016. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. Front. Microbiol. 7, 979.

Robert, S., Gysemans, C., Takiishi, T., Korf, H., Spagnuolo, I., Sebastiani, G., et al., 2014. Oral delivery of glutamic acid decarboxylase (GAD)-65 and IL10 by Lactococcus lactis reverses diabetes in recent-onset NOD mice. Diabetes 63 (8), 2876–2887.

Roberto, B.C., DC, M., Leone, L., Pedata, M., Meli, R., Calignano, A., 2011. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J. Gasteroenterol. 17 (12), 1519–1528.

Rosenbaum, J.T., Asquith, M., 2018. The microbiome and HLA-B27-associated acute anterior uveitis. Nat. Rev. Rheumatol. 14 (12), 704–713.

Saeidi, N., Wong, C.K., Lo, T.M., Nguyen, H.X., Ling, H., Leong, S.S., et al., 2011. Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen. Mol. Syst. Biol. 7 (521), 55.

Said, H.M., Nexo, E., 2018. Gastrointestinal handling of water-soluble vitamins. Compr. Physiol. 8 (4), 1291–1311.

Salminen, S., Collado, M.C., Endo, A., Hill, C., Lebeer, S., Quigley, E.M.M., et al., 2021. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat. Rev. Gastroenterol. Hepatol. 18 (9), 649–667, 2021/09/01.

Samaras, S.H.M., 2020. Skin Microbiome Handbook: From Basic Research to Product Development. Scrivener.

Santos, T.M., Ledbetter, E.C., Caixeta, L.S., Bicalho, M.L., Bicalho, R.C., 2011. Isolation and characterization of two bacteriophages with strong in vitro antimicrobial activity against *Pseudomonas aeruginosa* isolated from dogs with ocular infections. Am. J. Vet. Res. 72 (8), 1079–1086.

Saputo, S., Faustoferri, R.C., Quivey Jr., R.G., 2017. A drug repositioning approach reveals that Streptococcus mutans is susceptible to a diverse range of established antimicrobials and nonantibiotics. Antimicrob. Agents Chemother. 62 (1), 01674-17.

Sarkissian, C.N., Shao, Z., Blain, F., Peevers, R., Su, H., Heft, R., et al., 1999. A different approach to treatment of phenylketonuria: phenylalanine degradation with recombinant phenylalanine ammonia lyase. Proc. Natl. Acad. Sci. U S A 96 (5), 2339–2344.

Sassone-Corsi, M., Nuccio, S.-P., Liu, H., Hernandez, D., Vu, C.T., Takahashi, A.A., et al., 2016. Microcins mediate competition among Enterobacteriaceae in the inflamed gut. Nature 540 (7632), 280–283.

Scholz, C.F.P., Kilian, M., 2016. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus Propionibacterium to the proposed novel genera Acidipropionibacterium gen. nov., Cutibacterium gen. nov. and Pseudopropionibacterium gen. nov. Int. J. Syst. Evol. Microbiol. 66 (11), 4422–4432.
Schrezenmeir, J., de Vrese, M., 2001. Probiotics, prebiotics, and synbiotics–approaching a definition. Am. J. Clin. Nutr. 73 (2 Suppl. l).
Segata, N., Haake, S.K., Mannon, P., Lemon, K.P., Waldron, L., Gevers, D., et al., 2012. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genom. Biol. 13 (6), R42.
Sender, R., Fuchs, S., Milo, R., 2016. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 14 (8).
Shakya, M., Lo, C.-C., Chain, P.S.G., 2019. Advances and challenges in metatranscriptomic analysis. Front. Genet. 10. 2019-September-25.
Shankargouda, P., R, R.S., Amrutha, N., Sanketh, D.S., 2013. Oral microbial flora in health. World J. Dent. [Review] 4 (4), 262–266. October-December.
Sharma, P., Kumar, T., Yadav, M., Gill, S.S., Chauhan, N.S., 2021. Plant-microbe interactions for the sustainable agriculture and food security. Plant Gene 28, 100325. https://doi.org/10.1016/j.plgene.2021.100325. ISSN 2352-4073.
Shen, T.C., Albenberg, L., Bittinger, K., Chehoud, C., Chen, Y.Y., Judge, C.A., et al., 2015. Engineering the gut microbiota to treat hyperammonemia. J. Clin. Invest. 125 (7), 2841–2850.
Shi, Z., Li, S.K., Charoenputtakun, P., Liu, C.Y., Jasinski, D., Guo, P., 2018. RNA nanoparticle distribution and clearance in the eye after subconjunctival injection with and without thermosensitive hydrogels. J. Control Releas. 270, 14–22.
Shibagaki, N., Suda, W., Clavaud, C., Bastien, P., Takayasu, L., Iioka, E., et al., 2017. Aging-related changes in the diversity of women'sskin microbiomes associated with oral bacteria. Sci. Rep. 7 (1), 10567.
Singh, A., Hacini-Rachinel, F., Gosoniu, M.L., Bourdeau, T., Holvoet, S., Doucet-Ladeveze, R., et al., 2013. Immune-modulatory effect of probiotic Bifidobacterium lactis NCC2818 in individuals suffering from seasonal allergic rhinitis to grass pollen: an exploratory, randomized, placebo-controlled clinical trial. Euro. J. Clin. Nutri. 67 (2), 161–167.
Singh, P.K., Donovan, D.M., Kumar, A., 2014. Intravitreal injection of the chimeric phage endolysin Ply187 protects mice from *Staphylococcus aureus* endophthalmitis. Antimicrob. Agents Chemother. 58 (8), 4621–4629.
Slomka, V., Herrero, E.R., Boon, N., Bernaerts, K., Trivedi, H.M., Daep, C., et al., 2018. Oral prebiotics and the influence of environmental conditions in vitro. J. Periodontol. 89 (6), 708–717.
Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly, Y.M., et al., 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341 (6145), 569–573.
Smits, L.P., Bouter, K.E., de Vos, W.M., Borody, T.J., Nieuwdorp, M., 2013. Therapeutic potential of fecal microbiota transplantation. Gastroenterology 145 (5), 946–953.
Stearns, J.C., Davidson, C.J., McKeon, S., Whelan, F.J., Fontes, M.E., Schryvers, A.B., et al., 2015. Culture and molecular-based profiles show shifts in bacterial communities of the upper respiratory tract that occur with age. Isme J. 9 (5), 1246–1259.
Stinson, L.F., Boyce, M.C., Payne, M.S., Keelan, J.A., 2019. The not-so-sterile womb: evidence that the human fetus is exposed to bacteria prior to birth. Front. Microbiol. 10, 1124.
Strandwitz, P., 2018. Neurotransmitter modulation by the gut microbiota. Brain Res. 1693 (Pt B), 128–133.
Suzuki, T., Sutani, T., Nakai, H., Shirahige, K., Kinoshita, S., 2020. The microbiome of the meibum and ocular surface in healthy subjects. Invest. Ophthalmol. Vis. Sci. 61 (2), 18.
Takiishi, T., Korf, H., Van Belle, T.L., Robert, S., Grieco, F.A., Caluwaerts, S., et al., 2012. Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified Lactococcus lactis in mice. J. Clin. Invest. 122 (5), 1717–1725.
Tan, T.G., Sefik, E., Geva-Zatorsky, N., Kua, L., Naskar, D., Teng, F., et al., 2016. Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. Proc. Natl. Acad. Sci. U S A 113 (50), E8141–E8150.
Tanzer, J.M., Fisher, J., Freedman, M.L., 1982. Preemption of Streptococcus mutans 10449S colonization by its mutant 805. Infect. Immun. 35 (1), 138–142.

Teo, S.M., Mok, D., Pham, K., Kusel, M., Serralha, M., Troy, N., et al., 2015. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe. 17 (5), 704–715.

Tsai, Y.T., Cheng, P.C., Liao, J.W., Pan, T.M., 2010. Effect of the administration of Lactobacillus paracasei subsp. paracasei NTU 101 on Peyer's patch-mediated mucosal immunity. Int. Immunopharmacol. 10 (7), 791–798.

Tsilingiri, K., Rescigno, M., 2013. Postbiotics: what else? Benef. Microbe. 4 (1), 101–107.

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E.G., de Vos, W.M., et al., 2013. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. New England J. Med. 368 (5), 407–415.

Velmurugan, S., Gan, J.M., Rathod, K.S., Khambata, R.S., Ghosh, S.M., Hartley, A., et al., 2016. Dietary nitrate improves vascular function in patients with hypercholesterolemia: a randomized, double-blind, placebo-controlled study. Am. J. Clin. Nutr. 103 (1), 25–38.

Verma, M.K., Ahmed, V., Gupta, S., Kumah, J., Pandey, R., Mandhan, V., Chauhan, N.S., 2018. Functional metagenomics identifies novel genes *ABCTPP, TMSRP1* and *TLSRP1* among human gut enterotypes. Sci. Rep. 8 1397. https://doi.org/10.1038/s41598-018-19862-5.

Vieira, S.M., Hiltensperger, M., Kumar, V., Zegarra-Ruiz, D., Dehner, C., Khan, N., et al., 2018. Translocation of a gut pathobiont drives autoimmunity in mice and humans. Science 359 (6380), 1156–1161.

Wang, I.J., Wang, J.Y., 2015. Children with atopic dermatitis show clinical improvement after Lactobacillus exposure. Clin. Exp. Allergy 45 (4), 779–787.

Weinbauer, M.G., 2004. Ecology of prokaryotic viruses. FEMS Microbiol. Rev. 28 (2), 127–181.

Wen, X., Miao, L., Deng, Y., Bible, P.W., Hu, X., Zou, Y., et al., 2017. The influence of age and sex on ocular surface microbiota in healthy adults. Invest. Ophthalmol. Vis. Sci. 58 (14), 6030–6037.

Whelan, F.J., Verschoor, C.P., Stearns, J.C., Rossi, L., Luinstra, K., Loeb, M., et al., 2014. The loss of topography in the microbial communities of the upper respiratory tract in the elderly. Ann. Am. Thorac. Soc. 11 (4), 513–521.

Willcox, M.D., 2013. Characterization of the normal microbiota of the ocular surface. Exp. Eye Res. 117, 99–105.

Wylie, K.M., Mihindukulasuriya, K.A., Zhou, Y., Sodergren, E., Storch, G.A., Weinstock, G.M., 2014. Metagenomic analysis of double-stranded DNA viruses in healthy adults. BMC Biol. 12 (71), 014–0071.

Xu, L.Z., Yang, L.T., Qiu, S.Q., Yang, G., Luo, X.Q., Miao, B.P., et al., 2016. Combination of specific allergen and probiotics induces specific regulatory B cells and enhances specific immunotherapy effect on allergic rhinitis. Oncotarget 7 (34), 54360–54369.

Yadav, M., Chauhan, N.S., 2020. Overview of the rules of the microbial engagement in the gut microbiome: a step towards microbiome therapeutics. J. Appl. Microbiol. https://doi.org/10.1111/jam.14883.

Yadav, M., Chauhan, N.S., 2022. Microbiome therapeutics: exploring the present scenario and challenges. Gastroenterol. Rep. 10. https://doi.org/10.1093/gastro/goab046 goab046.

Yadav, M., Verma, M.K., Chauhan, N.S., 2018. A review of metabolic potential of human gut microbiome in human nutrition. Archiv. Microbiol. 200 (2), 203–217. https://doi.org/10.1007/s00203-017-1459-x.

Yadav, M., Pandey, R., Chauhan, N.S., 2020. Catabolic machinery of the human gut microbes bestow resilience against vanillin antimicrobial nature. Front. Microbiol. https://doi.org/10.3389/fmicb.2020.588545.

Yadav, M., Lomash, A., Kapoor, S., Pandey, R., Chauhan, N.S., 2021. Mapping of the benzoate metabolism by human gut microbiome indicates food-derived metagenome evolution. Sci Rep 11, 5561. https://doi.org/10.1038/s41598-021-84964-6.

Yadav, M., Kumar, T., Kanakan, A., Maurya, R., Pandey, R., Chauhan, N.S., 2022. Isolation and characterization of human intestinal bacteria *Cytobacillus oceanisediminis* NB2 for probiotic potential. Front. Microbiol. 13, 932795. https://doi.org/10.3389/fmicb.2022.932795.

Yan, M., Pamp, S.J., Fukuyama, J., Hwang, P.H., Cho, D.Y., Holmes, S., et al., 2013. Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and *S. aureus* carriage. Cell Host Microbe. 14 (6), 631–640.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., et al., 2012. Human gut microbiome viewed across age and geography. Nature 486 (7402), 222–227.

Ying, S., Zeng, D.-N., Chi, L., Tan, Y., Galzote, C., Cardona, C., et al., 2015. The influence of age and gender on skin-associated microbial communities in urban and rural human populations. PLoS ONE 10 (10), e0141842.

Yungareva, T., Urshev, Z., 2018. The aggregation-promoting factor in Lactobacillus delbrueckii ssp. bulgaricus: confirmation of the presence and expression of the apf gene and in silico analysis of the corresponding protein. World J. Microbiol. Biotechnol. 34 (7), 018–2480.

Zacharof, M.P., Lovitt, R.W., 2012. Bacteriocins produced by lactic acid bacteria a review article. APCBEE Procedia 2, 50–56.

Zhang, F., L, W., Shi, Y., Fan, Z., Ji, G., 2012. Should we standardize the 1,700-year-old fecal microbiota transplantation? Am. J. Gastroenterol. 107 (11), 1755.

Zhang, Y., Li, C.X., Zhang, X.Z., 2021. Bacteriophage-mediated modulation of microbiota for diseases treatment. Adv. Drug Deliv. Rev. 176 (113856), 5.

Zhao, Y.C., Bassiouni, A., Tanjararak, K., Vreugde, S., Wormald, P.J., Psaltis, A.J., 2018. Role of fungi in chronic rhinosinusitis through ITS sequencing. Laryngoscope 128 (1), 16–22.

Zhong, H., Penders, J., Shi, Z., Ren, H., Cai, K., Fang, C., et al., 2019. Impact of early events and lifestyle on the gut microbiota and metabolic phenotypes in young school-age children. Microbiome 7 (1), 2.

Zhou, Y., Mihindukulasuriya, K.A., Gao, H., La Rosa, P.S., Wylie, K.M., Martin, J.C., et al., 2014a. Exploration of bacterial community classes in major human habitats. Genom. Biol. 15 (5), 2014–2015.

Zhou, Y., Holland, M.J., Makalo, P., Joof, H., Roberts, C.H., Mabey, D.C., et al., 2014b. The conjunctival microbiome in health and trachomatous disease: a case control study. Genom. Med. 6 (11), 014–0099.

Zhou, J., Yang, J., Dai, M., Lin, D., Zhang, R., Liu, H., et al., 2020. A combination of inhibiting microglia activity and remodeling gut microenvironment suppresses the development and progression of experimental autoimmune uveitis. Biochem. Pharmacol. 180 (114108), 20.

Zhu, T., Liu, X., Kong, F.Q., Duan, Y.Y., Yee, A.L., Kim, M., et al., 2019. Age and mothers: potent influences of children's skin microbiota. J. Invest. Dermatol. 139 (12), 2497–2505.

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RESEARCH ARTICLE Open Access

A study to identify the practices of the buffalo keepers which inadvertently lead to the spread of brucellosis in Delhi

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Abstract

Background: India has the largest Buffalo population in the world, with every household in rural India owning buffaloes depending upon daily milk requirement – dairy farmers can own between 10 to 70 buffaloes. The health of Indian buffaloes is of economic importance since India is one of the largest buffalo meat exporters in the world, and Indian Buffalo semen is sold in the USA for breeding purposes. However, National Control Program on brucellosis is only active in South India and in Panjab (a North Indian state with high human brucellosis incidence). Our aim was to assess the knowledge and practices of the buffalo keepers of Delhi that make them susceptible to brucellosis.

Results: Amongst all the 11 districts of Delhi, there was 0% awareness about brucellosis and also about the S19 vaccine as the buffalo keepers had never heard of S19 vaccine which is available at minimal cost from Indian Veterinary Research Institute, Bareilly, India. Majority of the respondents drink raw milk, sleep in cattle sheds, do not isolate sick cattle, do not test buffaloes blood for any disease before purchasing them, apply intrauterine medication with bare hands to buffalo after abortion of foetus, never clean their cattle sheds with a disinfectant and believe that they can only acquire skin infections from cattle. All of these habits make them prone to brucellosis. While about 20 to 27% of respondents reported a history of abortions and retained placenta, disposed of the placenta with bare hands, and applied raw milk on cracked lips. It was surprising to note that majority of them never reared small ruminants like sheep and goat with buffaloes or *Bos* species as they were aware of the rapid spread of disease from small to big ruminants.

Conclusions: We found that buffalo keepers were ignorant of brucellosis, its causative agent, relevant vaccines and that they also involved in high-risk activities. As such, our findings highlight a need for buffalo keepers to be better educated via several awareness camps to minimize human exposure to *Brucella* in Delhi.

Keywords: Brucella, Delhi, Cattle keepers, Practices, Habits

Background

Brucellosis is a classified group III risk disease which has easy airborne transmission [6, 42]. *Brucella*, the causative agent of brucellosis is classified by Centers For Disease Control and Prevention or CDC as a category B pathogen that has the potential to be developed into a bioweapon. Brucellosis is endemic in India and affects dairy farming [19, 33, 40]. Bovine brucellosis has been discovered as the main reason for disease propagation in humans due to animal handling and consumption of bovine products [11]. Countries like Australia, Canada, Cyprus, Japan, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden and the United Kingdom have been able to eradicate human brucellosis because they have eradicated bovine brucellosis [7, 26]. Although some reliable reports are available from Western countries, brucellosis is always underreported in Asian countries. It has been identified that there is lack of data related to brucellosis from India, China and Sub-Saharan Africa [29].

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The world organization for animal health (OIE) defines standards for surveillance, diagnosis, epidemiology, control, eradication efforts, and the reduction of risk for animal health [16]. It means that all these factors must amalgamate to establish a better healthcare system. India has been identified as one of the hotspots for emerging infectious diseases and brucellosis is one of the emerging diseases [38]. New Delhi, the National Capital Territory of India, boasts of one of the best health care system in India. But as yet the website of the Development department of Delhi Government [http://delhi.gov.in/wps/wcm/connect/lib_development/Development/Home/Citizen+Charter] does not give any information about brucellosis. The diagnostic innovations are of no use if people of the country have no knowledge about the disease. Therefore, spreading awareness is the most important aspect of a control program. Although the brucellosis control program is very aggressive in South India [14, 28], but it is very frail in North India except in Punjab which is a North Indian state with a high incidence of brucellosis [5]. During our study, we observed that the majority of the dairy farmers reared exclusively buffaloes in Delhi. Many among them reared *Bos* species in fewer numbers (to fulfil the household requirement) than buffaloes on the same farm. The reason for this practice was explained to be the thicker milk quality along with the higher milk volumes derived from the buffaloes. As the spread of infectious diseases can be regulated by amending the practices and taking precautions thus we did a survey to analyze the practices of the buffalo keepers that may be furnishing the spread of brucellosis from the infected buffaloes. At the same time, we informed the buffalo keepers of the ways to rectify their animal handling so as to improve animal health and to curtail the spread of brucellosis.

Methods

Informed consent

All the participants signed a consent form prior to responding to the survey. The questionnaires were signed by the cattle keepers after filling them.

Sampling

A purposive sampling of the buffalo keepers was conducted from August 2015 to December 2016 in order to analyze their practices which promote the spread of brucellosis in the National Capital Territory of New Delhi, the capital city of India. As brucellosis is regarded as an occupational hazard, therefore, this study was conducted by interviewing the buffalo keepers of different districts of Delhi. The buffalo keepers come in close contact with the livestock therefore they constitute the high risk population for brucellosis. The buffalo keepers here refer to the human population of Delhi who reared buffalo exclusively for their household needs or for dairy farming. This human population also includes those who reared few *Bos* species on their cattle sheds to fulfil their household demands or for a few customers. This survey does not include data from the cattle keepers who exclusively reared domesticated cattle (*Bos* species) only. According to the census of 2012 there are 162,142 female and 20,445 male buffalos in rural and urban area of New Delhi. This includes the population of 11 districts of Delhi. The current census report is pending. We surveyed 1200 cattle sheds which included data of 5550 buffaloes (4828 females and 722 males) only. It is pin-pointed that the *Bos* species (usually 1 to 3 in number on each farm) on the surveyed cattle sheds were not included in this animal count. There is no report on the exact number of cattle sheds housing buffaloes per district in Delhi. Here, a 'cattle shed' is defined as the cattle establishment used to house buffaloes only or to house a few *Bos* species with buffaloes. These cattle establishments varied in the structure being close-house or open-house or some completely on the road-side or in the colony lanes between boundary walls of houses. The pictures of various cattle sheds are available as Additional files 1, 2, 3, 4 and 5. They do not resemble any buffalo farm advertised online by Indian companies as the common man cannot afford them. We tried to tap the maximum number of cattle sheds in each district but many people were not ready to interact. Therefore our study pertains to only those buffalo keepers who were ready to interact with the college students.

Questionnaire

A structured questionnaire was developed in English language and translated in the Hindi language (the native tongue of North Indians). The questionnaire contained both open-ended and close-ended questions. The face to face method of approach was employed to collect the data. The questionnaire is available as the supporting file. The questionnaire revolved around the issues affecting the spread of brucellosis like biosecurity, reproductive health of the livestock, maintenance of cattle sheds and knowledge about zoonoses. The issues addressed by the questionnaire and their relevance to brucellosis have been discussed in Table 1. Each buffalo keeper was asked the number of male and female buffalo housed in their cattle sheds, they were asked if they got their cattle vaccinated or not, if yes then they were asked to name the vaccines/ medicines they injected their cattle with. Each buffalo keeper was asked if their buffaloes were prone to miscarriages, if yes then in which month; they were asked if they separated sick animal from the healthy ones; they were also asked if they consumed unboiled/ unpasteurized milk; accessibility to veterinary doctors was also asked; buffalo keepers were asked how often do

Table 1 Questionnaire pertained to the matter of biosecurity, reproductive health of the livestock, maintenance of cattle sheds and knowledge of zoonoses

S.N.	Issue addressed	Relevance to Brucellosis
1.	Consumption of raw milk and Application of raw milk on cracked lips	Infected buffalo secrete large amounts of *Brucella* in their milk.
2.	Assisting animal birth, application of intrauterine medication post abortion, disposing aborted foetus and placenta with naked hands.	Uterine fluid, Placental membranes, aborted foetus of infected buffalo during parturition or abortion are rich sources of *Brucella*.
3.	History of abortion and retained placenta	The seroprevalence of brucellosis is found to be significantly higher in animals with a history of abortion and retained placenta.
4.	Knowledge about Brucellosis or any other zoonoses and S19 vaccine	Knowledge about a disease makes the high risk population cautious and thus prevents the spread. Vaccination of young animals is known to reduce burden of disease.
5.	Rearing small ruminants with large ruminants	Infectious diseases spillover from small ruminants to large ruminants and cause huge economic loss.
6.	Sleeping in cattle sheds	Close contact with buffalo is a risk factor identified for human Brucellosis.
7.	Blood testing before sale and purchase of cattle	Diagnosis of brucellosis may curb the sale of non-productive buffalo and curb the spread of disease.
8.	Separation of sick animals	Intermingling of sick buffalo or domesticated cattle like *Bos* sp. with healthy buffalo may facilitate the transmission of brucellosis to susceptible cattle.
9.	Use of disinfectant to clean the cattle shed	Disinfectants lyse the gram negative bacteria and thus remove infection from the environment of the cattle shed.

the Government Organizations come for blood tests. They were asked about the precautions they took while handling an aborted fetus. They were asked if the adult female buffaloes were milked; socio-economic data, medical histories. In order to receive an unbiased response, the disease of interest was not revealed to the respondents and the question regarding their knowledge about brucellosis was only asked at the end. On the completion of the questionnaire, the respondents were informed about brucellosis, safe livestock handling, S19 vaccinations and other measures to prevent the spread of brucellosis. Data validation was done during data collection in the field and also at the time of translation to English. Responses to the close-ended questions have been tabulated and responses to open-ended questions have been elaborated in result and discussion section. Data from questionnaires were entered in Microsoft Excel2010. "Yes" or "No" or "don't know" responses were recorded as per the response for each respondent. The 'countif' application was used to find the percentage of each response for each question.

Results

Income groups

As brucellosis is linked to the economic status of people, therefore, we enquired about the income of each respondent from districts tabulated in Table 2. Cattle sheds surveyed in New Delhi, Central Delhi, West Delhi and South East Delhi were fewer as these areas fall in the urban area with fewer cattle establishments. As is evident from Fig. 1, it can be seen that the 50% of the respondents belonged to the least income group of less than Rupees one Lac per annum, while rest of the respondents fell into higher slabs. It is reiterated here that 1 Lac rupees are equivalent to 1500 US$. It can be seen that a meagre percentage of 2.41% of respondents belonged to the category of people who earned more than 7 Lac rupees per annum. About 5.79% did not know their income. Thus it can be concluded that most people belonged to the lower income group in our survey.

Knowledge about brucellosis

As is evident from Table 3, 0% of respondents knew about brucellosis. Thus they were not cognizant of *Brucella* infections. In our study, it was observed that 37% of cattle keepers got their cattle vaccinated but only 9.25% of respondents could name any vaccine. The vaccines named by these respondents included anthrax vaccine, Foot and Mouth Disease or FMD vaccine, Enterotoxaemia or ET and Black Quarter or BQ vaccine. None of the respondents could name S19 or RB51 vaccine. Exactly 13% of respondents expressed that they do not know if their cattle is vaccinated or not. While 15% of respondents agreed that they only sometimes get their cattle vaccinated. Therefore it could be concluded that awareness about brucellosis was completely absent amongst the buffalo keepers. Majority of respondents in our study, 98%, conveyed that they did not rear small ruminants with buffaloes because when a disease affects one small ruminant then it spreads to all the big and small ruminants in the cattle sheds resulting in a huge loss. Thus it can be said that the risk of *Brucella* infection spilling from small ruminants to large ruminants was not significant amongst the population surveyed. Thus the buffalo keepers are conscious of the danger of the rapid spread of infection

Table 2 The survey rate of the buffalo keepers per district of Delhi, India

S.N.	District	Headquarter	Subdivisions	Number of cattlesheds surveyed
1	New Delhi	Connaught Place	Chanakyapuri, Delhi Cantonment, Vasant Vihar	20
2	North Delhi	Narela	Model Town, Narela, Alipur	164
3	North West Delhi	Kanjhawala	Rohini, Kanjhawala, Sarawati Vihar	150
4	West Delhi	Rajouri Garden	Patel Nagar, Panjabi Bagh, Rajouri Garden	85
5	South West Delhi	Dwarka	Dwarka, Najafgarh, Kapashera	171
6	South Delhi	Saket	Saket, Hauz Khas, Mehrauli	128
7	South East Delhi	Defence Colony	Defense Colony, Kalkaji, Sarita Vihar	34
8	Central Delhi	Daryaganj	Kotwali, Civil lines, Karol Bagh	42
9	North East Delhi	Seelampur	Seelampur, Yamuna Vihar, Karawal Nagar	128
10	Shahdara	Shahdara	Shahdara, Seemapuri, Vivek Vihar	150
11	East Delhi	Preet Vihar	Preet Vihar, Gandhi Nagar, Mayur Vihar	128

from small to large ruminants. It was observed that many buffalo keepers of Delhi kept dogs on the cattle shed as an alarm system to alert the owner of cattle thieves. Most cattle sheds were open house thus there is a high probability of the contamination of the feed and water by infected stray dogs also. Dogs in the cattle shed form a parallel reservoir of *Brucella* because dogs shed *Brucella* in reproductive fluids and spread bovine brucellosis [31]. Despite its endemism it was confounding to observe ignorance towards brucellosis, thus during our study, we apprised the buffalo keepers about brucellosis and S19 vaccination in detail.

Availability of the veterinary services

In an open-ended question, all the respondents were asked to summarize the state of the veterinary services available to them. We were dismayed to know that the veterinary services in all the districts of Delhi were extremely poor. All respondents revealed that there have never been any awareness camps regarding brucellosis in their area. It is reiterated that the website of the Development department of Delhi Government does not give any information about brucellosis. Respondents of our study from remote villages of South West Delhi divulged that the sweepers of government hospitals learn to deliver intravenous injections. Such sweepers act as veterinary doctors and visit the village in hope of mining money. Respondents also shared that these sweepers inject tetanus toxoid for every health problem and charge Rs.700 per buffalo. Thus, the respondents shared that in case of ill health they inject the buffaloes with antibiotics like terramycin by themselves without any veterinary intervention (Additional file 6). They also informed that they take the buffaloes to the hospital only during worst case scenario.

Practices

To assess if the practices of buffalo keepers increase the risk of *Brucella* infection, a number of questions were included in the questionnaire. It was found that 38% of respondents drank raw milk. These respondents agreed that they sometimes drank directly from the udders of cow or

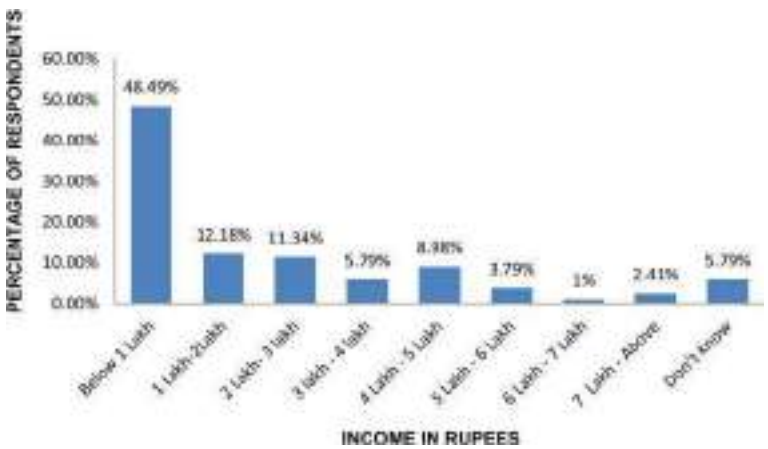

Fig. 1 Annual income of the respondents in Indian currency (Rupees)

Table 3 Knowledge and Practices of the buffalo keepers of Delhi, India

S.N.	Risk Factors	Yes %	No %	Sometimes %	Don't Know %
1	Drink raw milk	38	59	3	0
2	Milk the animal	60	35	5	0
3	Sleeping in Animal Sheds	79	11	10	0
4	Assisting Animal Birth	100	0	0	0
5	History of abortion in [3rd trimester] on farm	21	79	0	0
6	Disposed aborted fetus with naked hands	24	76	0	0
7	Incidence of retained placenta	20	70	10	0
8	Disposed placenta with naked hands	27	73	0	0
9	Applying raw milk on cracked lips	22	71	7	0
10	Vaccination of animals	37	35	15	13
11	Isolation of sick animals	32	68	0	0
12	Can you acquire disease from your cattle	37	38	25	0
13	Applied Intrauterine medication with naked hands after abortion	45	50	5	0
14	Use of disinfectant to clean cattle shed	13	85	2	0
15	Blood test before buying the animal	0	100	0	0
16	Do you rear goat and sheep with Buffalo and cow?	0	98	2	0
17	Have you heard of Brucellosis?	0	100	0	0

buffalo because they considered lactating animal as their religious mother. The practice of drinking raw milk driven by this belief puts these respondents at a risk of acquiring brucellosis. Respondents (65%) were also involved in milking the animal, 5% of respondents expressed that they sometimes milk the animal. As this activity involves touching the udders and coming in contact with raw milk thus this puts a large number of respondents at risk of acquiring brucellosis infection. Respondents from all the districts of Delhi revealed that their cattle sheds were prone to the theft of buffalo. Therefore, 79% of respondents always sleep in cattle sheds while 10% agreed that they only sometimes sleep in cattle sheds. *Brucella* can survive in soil for two to 6 months, therefore, sleeping in cattle sheds increases the probability of air-borne transmission or infection via an abrasion in the skin of buffalo keepers [23, 24]. All the respondents assisted the animal birth as it is celebrated as a special event in their family. Abortion in the 3rd trimester is a characteristic of brucellosis, and it was noted that 21% of surveyed cattle had abortions during this time. Thus it could be concluded that such buffaloes had the symptoms of brucellosis. Post-abortion the buffaloes are given tetanus toxoid intravenously only and no other medication was given. A low rate of abortion does not warrant a low prevalence rate as it is known that brucellosis may prevail as a silent disorder. Most buffalo keepers from Delhi confided that the buffaloes which suffered frequent abortions are sold off in local cattle fair. Thus such buffaloes stay in circulation as a major carrier of brucellosis. This practice will further deteriorate the state of affairs. We found that the aborted foetuses were disposed of with naked hands by only 24% of respondents as most of them call doctor under such situation. Cattle keepers in rural areas (22% of respondents) of Delhi used raw milk to heal cracked lips while in urban areas most cattle keepers (71% of respondents) applied commercially manufactured creams on cracked lips while 7% of respondents agreed that they applied raw milk on cracked lips only sometimes. Applying raw milk to heal cracked lips is an age-old traditional therapy in India which can also cause *Brucella* infection in humans. The incidence of retained placenta in buffaloes was reported by only 20% of respondents as 70% denied any retention and 10% expressed that this happened only sometimes. Isolation of sick animals was not being followed by 68% of respondents, thus these cattle keepers put their healthy cattle at a risk of the infectious diseases harboured by sick animals. Many respondents (37%) expressed that they could acquire some disease from their cattle, 25% of respondents said that they may sometimes acquire infection from cattle. These respondents suggested that the skin infections could be acquired from the cattle. Many respondents (38%) thought that they cannot acquire any infection from cattle. They also shared that even the sickness of the buffalo does not hinder them from consuming its milk. Drinking milk of a sick buffalo is a huge health hazard which cannot be avoided until the buffalo keepers are not convinced about the potential of disease communication from buffalo to them. At the end of the survey, we informed the buffalo keepers about the routes of disease communication from buffalo to humans.

Only 45% of respondents agreed that they applied intrauterine medication with naked hands post -abortion, 5% of respondents said that they only sometimes practiced this. While 50% of respondents expressed that they do not apply any medication post abortion. Application of intrauterine medication with naked hands post-abortion again is a practice which may communicate brucellosis from buffalo to the buffalo keepers. Assisting parturition exposes the buffalo keepers to fetal membranes, aborted fetus and uterine fluid contaminated with *Brucella* species [3, 17]. Only 13% of respondents expressed that they used disinfectant to clean the cattle shed, a meagre 2% agreed that they utilized disinfectant for this purpose. Exactly 85% of respondent agreed that they never used disinfectants to clean the cattle shed rather they washed the sheds with water and throw dry sand over the sheds to clean. In such an environment the propagation of several infectious diseases becomes most probable.

Discussion

Brucellosis has not been listed amongst the neglected tropical disease in India and South East Asia [22], but it has been identified as a neglected tropical disease by WHO, the World Health Organization [43]. Brucellosis is known to cause huge economic losses in India ranging between a loss of US $ 6.8 per cattle and US$18.2 per buffalo [37]. Brucellosis has been listed amongst zoonosis that affects the health of poor and affects the trade of animal products [41]. In our study, most of the respondents hailed from a poor background, therefore, it was pertinent to assess the economic status of the respondents of our survey. India comprises a big geographical entity, consequently, the epidemiology of brucellosis varies from one region to another. Seroprevalence varies from 3.3–11.4% in Chennai only, while Isloor et al reported overall prevalence for Karnataka to be 1.9% in cattle and 1.8% in buffalo. The Indian Agricultural Research Institute reported a 13.5% of stable endemic equilibrium for brucellosis in India [15, 30, 36]. Rahman et al. recognized that Delhi has the highest seroprevalence but the exact data was not published [30]. The Project Directorate on Animal Disease Monitoring and Surveillance (PDMAS, India) under the Ministry of Agriculture launched “Vision 2030” in 2011 [30] to eradicate brucellosis from India by the year 2030. The obligatory prerequisite for the success of any control program is building awareness about the disease. According to our study, the buffalo keepers from Delhi were totally unaware of brucellosis. The same level of awareness was reported from Kenya in the year 2007 and recently from Tajikistan [18, 21]. As opposed to this, the cattle keepers and shepherds from Egypt declared that their animals have a history of *Brucella* infection. They also confirmed that this infection was the main cause of abortions in their animals [10, 35]. Recent reports from Kenya show better awareness among the youngsters than the elderly. Better levels of awareness in recent years have been linked to higher seroprevalence of brucellosis in Kenya. Recent studies from other countries like Egypt, Tajikistan, and Kenya have reported that the livestock keepers were aware that brucellosis can spread from livestock to humans and that arthritis was a common symptom of the same [13, 21, 27]. In contrast to this, we found that cattle keepers from Delhi still think that only skin diseases can be acquired by handling cattle.

In western countries, there is a system of surveillance for brucellosis. Detailed data on brucellosis is compiled from time to time on demographics, the onset of symptoms, clinical signs, contact dates with the treating physicians, hospitalization, death, laboratory diagnosis, bacterial species, geographic origin and possible vehicle of infection. Standardized questionnaires containing these questions are sent to local health departments for every reported case of brucellosis. The same system needs to be developed in India as well. In terms of availability of information, India is 65 years behind the western countries [4, 12, 16, 32, 34]. Several countries identify their failures in controlling brucellosis [2] and discuss better ways along with newer possibilities to pin it down. This kind of model needs to be adopted by India as well. Other countries like the Gambia that report low prevalence for brucellosis should also be looked upon as a paradigm [9].

According to our study, the veterinary services in the rural areas of Delhi were not appropriate. Thus the buffalo keepers like to inject drugs by themselves in buffalo. Only when the problem escalates they take buffaloes to the remote veterinary hospitals. Studies from other countries like Egypt and Tajikistan also report reluctance on the part of the livestock keepers to contact veterinarians [11, 21]. It is also known that local dairies of India owned by the cattle keepers sell unpasteurized milk only. There have been reports of sale and purchase of unpasteurized dairy products in Iran, Egypt, Tajikistan, Uzbekistan, and Yemen also. This practice has been regarded as a prime risk factor for spread of human brucellosis [1, 8, 11, 39]. It is believed that even a sporadic abortion must be linked to brucellosis [3, 24] and the animal undergoing abortion must be culled. Many countries like India have reported the lack of official culling of infected sheep, goats, and buffalos as the main cause of high *Brucella* seropositivity [11]. Livestock keepers from other countries have reported that they feed the aborted foetuses to dogs or throw it in the water canal which adds to the spread of brucellosis [7]. This treacherous practice exposes the entire ecosystem to brucellosis. This habit was not reported

by the buffalo keepers from Delhi. Studies from other parts of the world also report that gloves and masks are not being utilized while handling aborted foetuses and while assisting parturition [11, 20, 21]. All these practices make the spread of brucellosis convenient and rampant.

Most of the underdeveloped countries across the globe face the same situation but a developed country like the United States of America (USA) has identified brucellosis as a prioritized zoonoses. Furthermore, they have made a road map to combat not only the zoonoses but also the infectious diseases by gauging their own capabilities on the scale of surveillance and availability of diagnostics. Despite being a developed country, the USA has revealed an insufficiency of diagnostic capability [25]. Though the focus of our survey was brucellosis, we can conclude that the knowledge about most infectious diseases was insufficient, also the practices of these cattle keepers put them at high risk of acquiring infectious diseases. Thus, like developed countries, India must adopt a holistic approach to combat zoonoses and other infectious diseases as a whole. Our study may be regarded as only an elementary research due to several limitations. Our major limitation is the sampling bias. There is no report on the total number of cattle sheds in all the districts of Delhi. Therefore, it is impossible to pinpoint the percentage of the cattle keepers from Delhi who harbour the same opinion or perform the same practices documented in this study. Though, we tried to overcome this limitation by interviewing as many buffalo keepers as were ready to interact with us. Another limitation of this study has been the summation of responses of cattle keepers from household cattle sheds, small cattle sheds, and large cattle sheds together. As the situation and configuration of these cattle sheds differ, therefore, this may also be having a confounding effect on the inference.

Conclusion

In the NCT of Delhi, we found that the cattle keepers have never been surveyed for their opinions and practices regarding any infectious disease. On interviewing the cattle keepers we realized that they are sensitive towards the medical needs of their cattle but they do not have appropriate help. Our study indicates that the cattle keepers are oblivious to the practices which cause spillover infection of *brucella* from their cattle to them. It can be concluded from our survey that the cattle farmers are ignorant of brucellosis, its causative agent, the route of its transmission, its symptoms and vaccination. The emotional and religious belief of the cattle keepers further exposes them to the threat of brucellosis. The absence of culling, free cattle trade and absence of blood testing before buying the cattle makes the situation even gross. Thus we endeavoured to enlighten the cattle keepers with the harmful husbandry practices which endorse the spread of brucellosis. The major problem faced by the control programs includes opinions of people and it is important to mould these opinions if brucellosis is to be combated successfully. In the current scenario, it is pertinent that the Indian Government must organize awareness camps before brucellosis becomes a bigger menace.

Additional files

Additional file 1: a) Open house cattle shed in North East Delhi b) and c) Open house cattle sheds of the South West Delhi district. (PPTX 816 kb)

Additional file 2: A road-side establishment of North Delhi district. (PNG 627 kb)

Additional file 3: One side open cattle shed used for housing buffalo and *Bos* species together. (PNG 561 kb)

Additional file 4: Close house cattle shed in Shahdara district, arrow shows the opening of the housing area. This cattle shed was completely dark with light going through this opening. (PNG 373 kb)

Additional file 5: Buffalo residing in the lane between houses in the South Delhi district. (PNG 324 kb)

Additional file 6: One of the medicines shown by the respondents was Terramycin, Injectable solution. (PNG 242 kb)

Additional file 7: Questionnaire. (DOCX 12 kb)

Abbreviations

BQ: Black Quarter; CDC: Centers For Disease Control and Prevention; ET: Enterotoxaemia; FMD: Foot and Mouth Disease; OIE: Organization for animal health; PDMAS: Project Directorate on Animal Disease Monitoring and Surveillance; USA: United States of America; WHO: World Health Organization

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Availability of data and materials

The survey questionnaire is available as the Additional file 7 of this manuscript. All relevant raw data will be freely available to any scientist wishing to use them for non-commercial purposes without breaching participant confidentiality.

Authors' contributions

PK, NK and RB designed the work. PK, AM, DS, AD, RM, S., RA, AM, MT, PA, SS analyzed the data. AD, RM and RM helped in the preparation of figure and tables. PK and RS organized the references. PK wrote the manuscript and critically revised the content. All the authors conducted the survey. All the authors have read and approved the manuscript.

Ethics approval and consent to participate

All respondents signed consent to participate in the survey. The research published here was approved by the Institutional Bioethical and Biosafety Committee of Shivaji College, University of Delhi. Reference number of the same is SHC308, Innovation Project cycle 2015/2016.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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References

1. Al-Shamahy HA, Whitty CJM, Wright SG. Risk factors for human brucellosis in Yemen: case control study. Epidemiol Infect. 2000;125:309–13.
2. Azzam RA, El-Gamal AM, Elsheemy MT. Failure of control of Brucella melitensis infection in a dairy herd. Assiut Vet Med J. 2009;55(121):274–85.
3. Crawford RP, Huber JD, Adams BC. Epidemiology and surveillance. In: Nielsen K, Duncan JR, editors. Animal brucellosis. Boca Raton: CRC Press; 1990. p. 131–48.
4. Dahouk SA, Neubauer H, Hensel A, Schöneberg I, Nöckler K, Alpers K, Merzenich H, Stark K, Jansen A. Changing epidemiology of human brucellosis, Germany. Emerg Infect Dis. 2007;13:1962–2005.
5. Dhand NK, Gumber S, Singh BB, Aradhana BMS, Kumar H, Sharma DR, Singh J, Sandhu KS. A study on the epidemiology of brucellosis in Punjab (India) using Survey Toolbox. Rev Sci Tech. 2005;24(3):879–85.
6. Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, Zinsstag J. Clinical manifestations of human brucellosis: a systematic review and metaanalysis. PLoS Negl Trop Dis. 2012;12:e1929.
7. Díaz AE. Epidemiology of brucellosis in domestic animals caused by *Brucella melitensis, Brucella suis* and *Brucella abortus*. Sci Tech Rev. 2013;32:53–60.
8. Earhart K, Vafakolov S, Yarmohamedova N, Michael A, Tjaden A, Soliman A. Risk factors of human brucellosis in Samarqand oblast, Uzbekistan. Int J Infect Dis. 2009;13:749–53.
9. Germeraad EA, Hogerwerf L, Faye-Joof T, Goossens B, van der Hoek W, Jeng M, Lamin M, Manneh IL, Nwakanma D, Roest HI, Secka A, Stegeman A, Wegmüller R, van der Sande MA, Secka O. Low Seroprevalence of Brucellosis in Humans and Small Ruminants in the Gambia. PLoS One. 2016; 11(11):e0166035.
10. Hegazy YM, Moawad A, Osman S, Ridler A, Guitian J. Ruminant brucellosis in the Kafr El sheikh governorate of the Nile Delta, Egypt: prevalence of a neglected zoonosis. PLoS Negl Trop Dis. 2011;5:e944.
11. Hegazy Y, Elmonir W, Hamid NHA, Elbauomy EM. Seroprevalence and "knowledge, attitudes and practices" (KAP) survey of endemic ovine brucellosis in Egypt. Acta Vet Scand. 2016;58:1.
12. Hernández-Mora G, Bonilla-Montoya R, Barrantes-Granados O, Esquivel-Suárez A, Montero-Caballero D, González-Barrientos R, Fallas-Monge Z, Palacios-Alfaro JD, Baldi M, Campos E, Chanto G, Barquero-Calvo E, Chacón-Díaz C, Chaves-Olarte E, Guzmán Verri C, Romero-Zúñiga JJ, Moreno E. Brucellosis in mammals of Costa Rica: an epidemiological survey. PLoS One. 2017;12(8):e0182644.
13. Holt H, Eltholth M, Hegazy Y, El-Tras W, Tayel A, Guitian J. *Brucella* spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health. 2011;11:341.
14. Hundal JS, Sodhi SS, Gupta A, Singh J, Chahal US. Awareness, knowledge, and risks of zoonotic diseases among livestock farmers in Punjab. Vet World. 2016;9(2):186–91.
15. Isloor S, Renukaradhya GJ, Rajasekhar M. A serological survey of bovine brucellosis in India. Sci Tech Rev. 1998;17(3):781–5.
16. Jebara KB. Surveillance, detection and response: managing emerging diseases at national and international levels. Sci Tech Rev. 2004;23(2):709–15.
17. John K, Fitzpatrick J, French N, Kazwala R, Kambarage D, Mfinanga GS, Macmillan A, Cleveland S. Quantifying risk factors for human brucellosis in rural northern Tanzania. PLoS One. 2010;5:e9968.
18. Kang' Ethe EK, Ekuttan CE, Kimani VN, Kiragu MW. Investigations into the prevalence of bovine brucellosis and the risk factors that predispose humans to infection among urban dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya. East Afr Med J. 2007;84:96–100.
19. Khurana SK, Srivastava SK, Prabhudas K. Seroprevalence of bovine brucellosis in Haryana by avidin-biotin serum ELISA and its comparison with RBPT and SAT. Indian J Anim Sci. 2012;82:448–50.
20. Kozukeev TB, Ajeilat S, Maes E, Favorov M. Risk factors for brucellosis-Leylek and Kadamay districts, Batken oblast, Kyrgystan, January-November. Morb Mortal Wkly Rep. 2006;28:31–4.
21. Lindahl E, Sattorov N, Boqvist S, Magnusson U. A study of knowledge, attitudes and practices relating to brucellosis among small scale dairy farmers in an urban and peri-urban area of Tajikistan. PLoS One. 2015;10:e0117318.
22. Lobo DA, Velayudhan R, Chatterjee P, Kohli H, Hotez PJ. The neglected tropical diseases of India and South Asia: review of heir prevalence, distribution, and control or elimination. PLoS Negl Trop Dis. 2011;5:e1222.
23. Mangalgi SS, Sajjan AG, Mohite ST, Gajul SJ. Brucellosis in Occupationally Exposed Groups. J Clin Diagn Res. 2016;10(4):DC24–7.
24. Makita K, Fevre EM, Waiswa C, Eisler MC, Thrusfield M, Welburn SC. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. BMC Vet Res. 2011;7:60.
25. Maxwell MJ, Freire de Carvalho MH, Hoet AE, Vigilato MA, Pompei JC, Cosivi O, Del Rio Vilas VJ. Building the road to a regional zoonoses strategy: a survey of zoonoses programmes in the Americas. PLoS One. 2017;12(3):e0174175.
26. Mohamed NS, Stephen MB, Nammalwar S. Brucellosis: a re-emerging zoonosis. Vet Microbiol. 2010;140:392–8.
27. Njuguna JN, Gicheru MM, Kamau LM, Mbatha PM. Incidence and knowledge of brucellosis in Kahuro district, Murang'a county, Kenya. Trop Anim Health Prod. 2017;49:1035–40.
28. Patil DP, Ajantha GS, Shubhada C, Jain PA, Kalabhavi A, Shetty PC, Hosamani M, Appannanavar S, Kulkarni RD. Trend of human brucellosis over a decade at tertiary care Centre in North Karnataka. Indian J Med Microbiol. 2016;34(4):427–32.
29. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis. 2006;6(2):91–9.
30. Rahman H. DBT Network Project on Brucellosis: Indian Council of Agricultural Research, Project Monitoring Unit, Project Directorate on Animal Disease Monitoring and Surveillance, Annual Report; 2013. https://icar.org.in/files/Vision%202030_PDADMAS-11-01-2012.pdf.
31. Prior MG. Isolation of Brucella abortus from two dogs in contact with bovine brucellosis. Can J Comp Med. 1976;40:117–8.
32. Rana UV, Sehgal S, Bhardwaj M. A sero-epidemiological study of brucellosis among workers of veterinary hospital and slaughter houses of union territory of Delhi. Int J Zoonoses. 1985;12(1):74–9.
33. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet Microbiol. 2002;90:183–95.
34. Russo G, Pasquali P, Nenova R, Alexandrov T, Ralchev S, Vullo V, Rezza G, Kantardjiev T. Reemergence of human and animal brucellosis, Bulgaria. Emerg Infect Dis. 2009;15:314–6.
35. Hassan S, Meshref AR, Khoudair RM, Ashour HM. Multicenter study of brucellosis in Egypt. Emerg Infect Dis. 2008;14:1916–8.
36. Senthil NR, Narayanan SA. Seroprevalence study of bovine brucellosis in slaughter house. Int J Adv Vet Sci Technol. 2013;2(1):61–3.
37. Singh BB, Dhand NK, Gill JP. Economic losses occurring due to brucellosis in Indian livestock populations. Prev Vet Med. 2015;119(3–4):211–5.
38. Singh BB, Sharma R, Gill JPS, Aulakh RS, Banga HS. Climate change, zoonoses and India. Sci Tech Rev. 2011;30:779–88.
39. Sofian M, Aghakhani A, Velayati AA, Banifazl M, Elsamifar A, et al. Risk factors for human brucellosis in Iran: a case-control study. Int J Infect Dis. 2008;12: 157–61.
40. Trangadia B, Rana SK, Mukharjee F, Srinivasan VA. Prevalence of brucellosis and infectious bovine rhinotrachitis in organized dairy farms in India. Trop Anim Health Prod. 2010;42:203–7.
41. Thiermann A. Emerging diseases and implications for global trade. Sci Tech Rev. 2004;23(2):701–8.
42. World Health Organization (WHO), 2006. The control of neglected diseases. A route to poverty alleviation. Report of a joint WHO/DFID-AHP meeting with the participation of FAO and OIE. [http://www.who.int/zoonoses/Report_Sept06.pdf].
43. World Health Organization. The control of neglected zoonotic diseases. Geneva: report of a joint WHO/DFID-AHP; 2005. p. 54. Available: http://www.who.int/zoonoses/Report_Sept06.pdf. Accessed 28 May 2010