Current Microbiology (2023) 80:109
https://doi.org/10.1007/s00284-023-03203-5

SHORT COMMUNICATION

The Influence of Probiotics in Reducing Cisplatin-Induced Toxicity in Zebrafish (*Danio rerio*)

Kusuma Kandati[1,2] · Ankireddy Seshadri Reddy[1] · John Sushma Nannepaga[2] · Buddolla Viswanath[1]

Received: 13 June 2022 / Accepted: 25 January 2023

Abstract

In this work, the effects of probiotic supplementation on cisplatin toxicity in zebrafish (*Danio rerio*) were examined. For this study, adult female zebrafish were given cisplatin (G2), the probiotic, *Bacillus megaterium* (G3), and cisplatin+*B. megaterium* (G4) for 30 days, in addition to the control (G1). In order to investigate changes in antioxidative enzymes, ROS production, and histological changes after treatment, the intestines and ovaries were excised. The levels of lipid peroxidation, glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase were found to be significantly higher in the cisplatin group than in the control group in both the intestine and the ovaries. Administration of the probiotic and cisplatin effectively reversed this damage. Histopathological analyses showed that the cisplatin group had much more damage than the control group and that probiotic+cisplatin treatment significantly cured these damages. It opens the door to probiotics being combined with cancer-related drugs, which may be a more efficient approach for minimizing side effects. The underlying molecular mechanisms of probiotics must be further investigated.

Introduction

The burden of cancer is substantial and is increasing globally. Cisplatin, an antineoplastic drug, is the only one that the FDA has approved for the treatment of advanced cancers. This drug is well-known for its usage in the treatment of many solid tumours as well as various forms of cancer. It exerts anticancer activity through a variety of mechanisms, but its preferred mechanism includes the generation of DNA lesions through interacting with purine bases on DNA, blocking the production of DNA, mRNA, and proteins, arresting DNA replication, followed by the activation of numerous signal transduction pathways that ultimately result in the death of cancer cells [1]. Cisplatin is utilized in the treatment of cancer despite its inherent issues with side effects and drug resistance, which include major kidney difficulties, allergic responses, poor immunity to infections, gastrointestinal problems, haemorrhaging, and hearing loss, particularly in younger patients [2]. Three key pathways can lead to the development of drug resistance: greater DNA repair by the cell of platinum-induced damage, lower drug absorption into cancer cells, and enhanced drug degradation and deactivation before it reaches nuclear DNA. The primary lethal mechanism of cisplatin in proliferating (cancer) cells is DNA binding; nephrotoxicity and ototoxicity appear to be caused by hazardous levels of ROS and protein dysregulation in multiple cellular compartments [3]. The therapeutic limitations of cisplatin have led to the development of numerous cisplatin analogs. Only two, oxaliplatin and carboplatin, have received unanimity of opinion. But compared to cisplatin, the majority of platinum compounds don't seem to offer much of a benefit. Although soft nucleophiles like glutathione and other proteins and peptides containing cysteine or methionine sulphur residues are easily bound by platinum-based drugs, nuclear DNA is their primary target [4, 5]. Although the precise mechanism of cisplatin's cytotoxicity is unknown, it mainly causes toxicity by increasing the generation of ROS, decreasing endogenous antioxidant defences, and promoting inflammation [6].

✉ John Sushma Nannepaga
johnsushma@gmail.com

✉ Buddolla Viswanath
drbuddollav@drbuddolla.org

[1] Dr. Buddolla's Institute of Life Sciences, Tirupati 517503, India

[2] Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati 517 502, India

Published online: 18 February 2023

Since there is currently no alternative therapy for drugs based on platinum, we believe that better side effect management is more important than the development of new drugs. Probiotics can therefore be supplemented with platinum-based drugs to lessen the effects, and their efficacy justifies investigation. Additionally, probiotics are recommended since they have less of an impact on healthy tissue than synthetic drugs [7]. Further, anti-inflammatory cytokines, which are crucial in preventing carcinogenesis, can be produced by probiotic bacteria more or less depending on the circumstances [1]. Since the production of ROS is the main cause of all adverse effects of platinum-based drugs, the development of potential ways to lower ROS levels or to lessen their effects may have a substantial impact on the negative effects. Numerous studies in recent years have added to our knowledge of the antioxidant potential of probiotics [8, 9]. Tissue damage is also frequently attributed to oxygen-derived free radicals. One of the many biological impacts of probiotics is the prevention of cancer. They significantly influence numerous other areas as well. Research on probiotics' ability to prevent or treat tumours is extensive. Recent studies have shown that probiotics help improve homeostasis and reduce the negative effects of chemotherapy [10–12]. Therefore, in the current study, we aim to assess how probiotics affect the reduction of cisplatin-induced toxicity in the Zebrafish model. The probiotic, *Bacillus megaterium* is used in a study for the first time to reduce the toxicity of cisplatin since it produces a variety of enzymes and has a high level of resistance to pathogenic bacteria.

Materials and Methods

Experimental Strains and Animals

The probiotic strain chosen was *Bacillus megaterium* de Bary (ATCC® 14581TM), and adult zebrafish were obtained from a local supplier. In this study, adult female zebrafish were used, and the fish were divided into four groups ($n = 10$). The fish water tank temperature was maintained at 25.0±0.5 °C, and they were kept on a 12-h/12-h light/dark cycle.

Experimental Groups

Group (a)—served as a control, fed solely a commercial diet (Tanganyika, freeze-dried tubifex worms).

Group (b)—Treated with probiotics (*B. megaterium* via the rearing water at a concentration of 10^6 CFU) by semi-static method, with the solution being replaced after 24 h [8].

Group (c)—Cisplatin (1 mg/kg) injected by intraperitoneal (IP) injection in total, four times—once every week—during the 30-day course of treatment. The following concentrations of cisplatin were selected for LC_{50} determination: 0.5, 1.0, 1.5, 2.0, and 2.5 mg/kg.

Group (d)—Probiotic + cisplatin of fishes was fed with a commercial diet along with the treatment.

Groups ($n = 10$)	Dosage	Treatment	No. of days
Control (a)	Only feed	None	30 days
Probiotic (b)	*B. megaterium* via the rearing water at a concentration of 10^6 CFU	Daily	30 days
Cisplatin (c)	Cisplatin (1 mg/kg) injected by intraperitoneal (IP	Once a week	30 days
Probiotic+cisplatin (d)	Probiotic—10^6 CFU via rearing water + cisplatin (1 mg/kg)	*B. megaterium*—daily; Cisplatin—Once a week	30 days

To assess an organism's susceptibility and probability of surviving in the presence of the drug cisplatin, the 96-h (hour post-fertilization-hpf) lethal concentration (LC50) tests were used. The water temperature, pH, dissolved oxygen concentration in each test tank, fish behavior, and mortality rate were all monitored on a daily basis during the treatment. Fish were euthanized by a lethal dosage of anesthetic (500 mg l-1 MS-222 [3-aminobenzoic acid ethyl ester]) after 30 days of treatment. The total wet weight of each fish was determined. Intestines and ovaries were sampled and immediately flash-frozen using liquid nitrogen and stored at −80 °C for ROS scavenging and antioxidant activity. For histopathology analyses, three-five of each group's intestines and ovaries were excised and preserved in 10% formalin. A petri dish containing MRS agar was first spread with the probiotic *B. megaterium*, then blank discs were inserted. The blank discs were administered cisplatin at concentrations of 25, 50, 75, and 100 µg/mL, and the plate was then incubated for 24 h at 37 °C; to assess the substantiating the interaction between cisplatin and probiotics. If zones develop, cisplatin is preventing the probiotic *B. megaterium* from growing; otherwise, a symbiotic interaction exists.

Antioxidant Enzyme Assays

ROS Scavenging Activity

The reactive oxygen species (ROS) like superoxide anion, peroxide, and hydroxyl radicals can be estimated using the quinol oxidation followed by the formation of corresponding adduct approach in the presence of intracellular ROS upon treatment of quinol with the cells. Initially, the percentage of ROS activity was investigated with hydrogen peroxide (H_2O_2) as a standard curve described in the protocol [13]. The percentage of ROS activity has been calculated using the following expression.

$$\text{TheROSscavengingactivitypercentage} = \frac{A_{C-A_T}}{A_C} \times 100, \quad (1)$$

where A_C = Absorbance of control, A_T = Absorbance of the sample.

Lipid Peroxidation

Malondialdehyde (MDA) is an end product of lipid peroxidation, which was measured in tissue homogenates of various groups based on the reaction with thiobarbituric acid (TBA) to form a pink color complex, MDA produced was determined with the absorbance coefficient of the MDA-TBA complex at 550 nm using 1, 1, 3,3-tetraethoxypropane (TMP) as the standard [14].

Glutathione Peroxidase and Glutathione Reductase

By catalysis conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) for the consumption of NADPH, the catalytic activity of glutathione peroxidase and glutathione reductase was measured spectrophotometrically at 340 nm. The specific activities were expressed as the nmol of NADPH consumption per min per mg of protein [15].

Superoxide Dismutase

The activity of superoxide dismutase was determined in the tissue homogenates by the modified method of NADH-phenazine methosulphate-nitro blue tetrazolium formazan inhibition reaction spectrophotometrically, measured at 550 nm [16].

Catalase

The catalytic activity of catalase was determined by a spectrophotometrical measurement of H_2O_2 breakdown at 240 nm. The specific activity of the enzyme was expressed as μmol of decomposed H_2O_2 per min per mg of protein [17].

Histopathology

According to the procedure of [18], tissue samples that were stored in 10% formalin were analyzed for histopathology investigation. The OLYMPUS CKX523 inverted microscope was used to take the images at 20×magnification.

Statistical Analysis

Data were expressed as mean±SD from at least three independent experiments. One-way ANOVA was performed for the comparison of results between untreated control and treatments. Values were considered significant when P values were < 0.05 and < 0.01.

Results and Discussion

Different morphological parameters were assessed after 30 days of therapy, and total wet weight was determined for all fish. As shown in Table S1, the probiotic group gained weight, the cisplatin group lost weight, the probiotic+cisplatin-treated group maintained their weight, and the cisplatin-treated group increased their body length. According to body mass index (BMI), control, the probiotic, probiotic+cisplatin-treated group was considered healthy with 0.1 g/cm^2 whereas, the cisplatin-treated group is considered unhealthy with 0.06 g/cm^2 of BMI. Probiotics can influence the health of the host in several ways: secreting secondary metabolites that inhibit the growth of microbial pathogens and/or directly stimulating immune responses to downregulate gut inflammation [19]. To assess the potential health benefits of live probiotics it is important to understand their optimal environment inside the host. Probiotic-host interaction was addressed using zebrafish. As shown in Fig. S1, cisplatin and the probiotic had a symbiotic connection, meaning that the drug did not inhibit the probiotic's growth. At 96 hpf, mortality tests on zebrafish exposed to different dosages of cisplatin revealed that the LC_{50} of cisplatin was 0.26 mg L^{-1}.

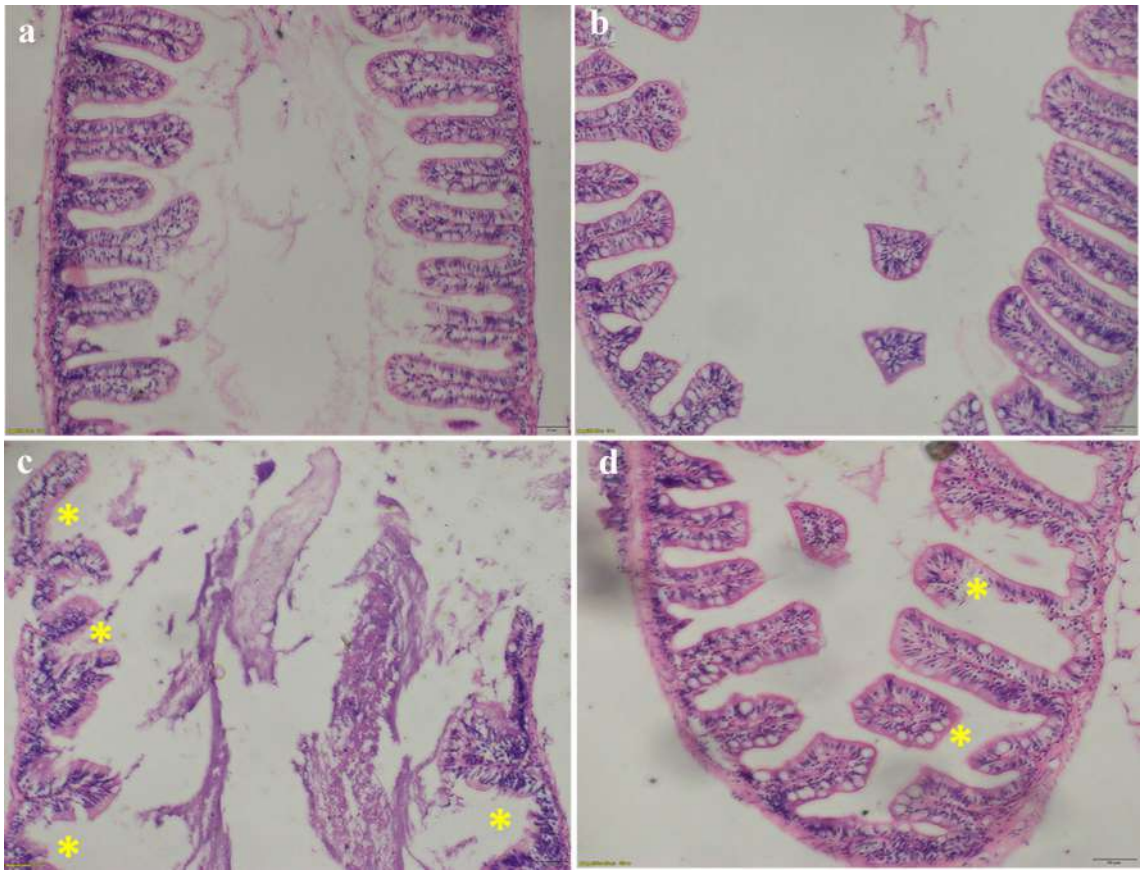

Fig. 1 Protective effects of probiotics on the histology of the intestine in cisplatin-induced zebrafish **a** control, **b** probiotic, **c** cisplatin, **d** cisplatin+probiotic groups. (*) indicates the damage intended to that particular region. Histopathological abnormalities in the intestines were examined using hematoxylin and eosin staining. In contrast to the usual architecture exhibited in the intestine of control fish, vacuolated gaps were observed in the cisplatin-intoxicated Intestine. Cisplatin-treated tissues lose the major intact basement membranes surrounding the intestines

The epithelial barrier in the control group's intestine was unaffected and showed no evidence of deterioration. The epithelial barrier in the intestines exposed to the probiotic *B. megaterium* was intact, there was no cell debris in the lumen, and there was no evidence of injury. The intestine was mostly homogeneous, columnar, polarized epithelia with an apical brush border, lateral cell border, and basal basement membrane at this stage. Columnar-shaped enterocytes were joined apically in both control and treated groups (Fig. 1) by a complex set of junctional complexes (tight junctions, adherent junctions, and desmosomes) that restrict membrane component movement between apical and basolateral cellular domains and serve as a barrier to the paracellular space through which luminal contents might otherwise enter the organism.

Chemotherapeutic agents have long been known to have toxic effects on ovarian function. When compared to cisplatin treatment alone, Cisplatin+probiotic administration resulted in more morphologically normal follicles and cell proliferation, lower apoptosis, and lower ROS production. Under normal physiological conditions, cells regulate reactive oxygen species levels by balancing reactive oxygen species production with scavenging system elimination (reduced glutathione-GSH, superoxide dismutase-SOD, and catalase-CAT). Ovaries from the control group showed normal ovarian morphology with centrally located oocytes and granulosa cells surrounded by normal intact basement

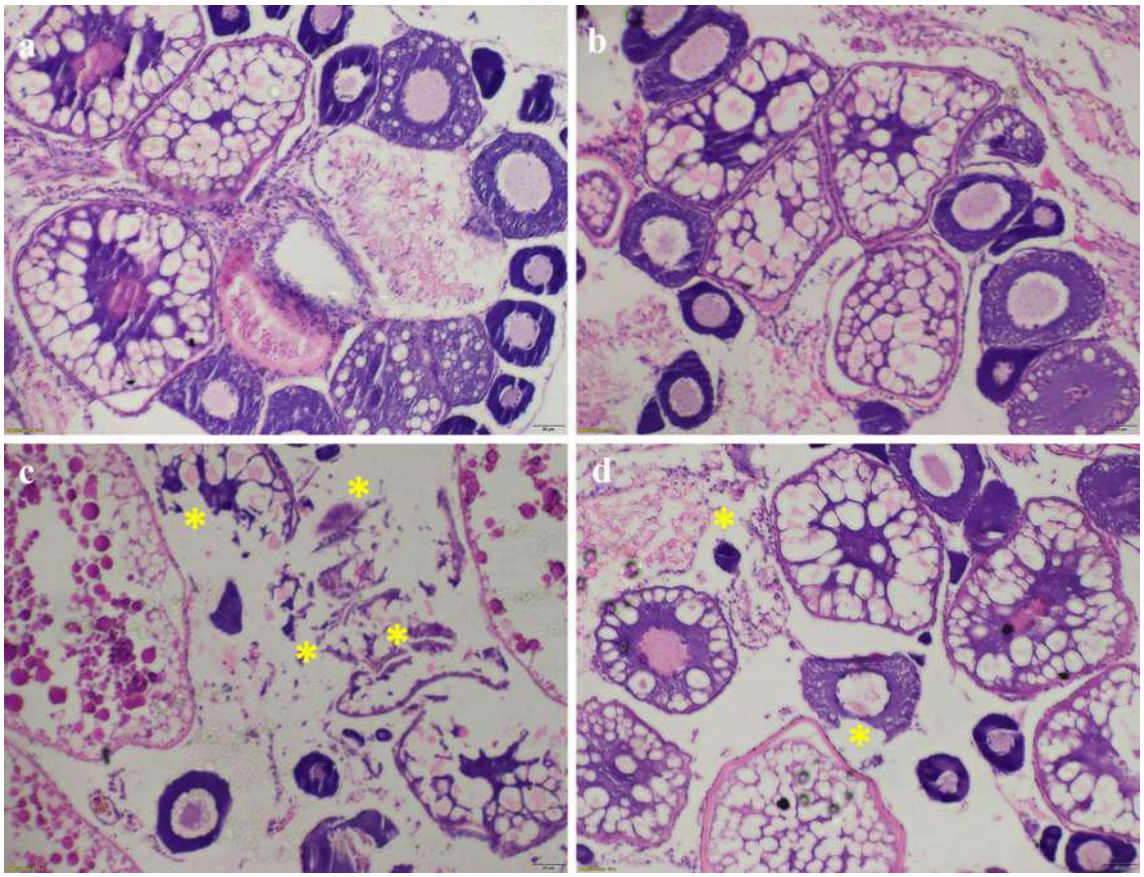

Fig. 2 Protective effects of probiotics on the histology of the ovary in cisplatin-induced zebrafish **a** control, **b** probiotic, **c** cisplatin, **d** cisplatin+probiotic groups. (*) indicates the damage intended to that particular region. Hematoxylin–eosin staining was used to look for histopathological changes in the ovaries. Oocytes and granulosa cells were centrally located and surrounded by normal intact basement membranes in the control group's ovaries. The cisplatin group, on the other hand, caused ovarian damage and a decrease in follicle number. When compared to the cisplatin group of fishes, cisplatin+probiotic treatment ameliorated minor abnormalities in the ovaries

membranes. However, the cisplatin group damaged the ovarian structure and promoted a reduction in the number of follicles. Cisplatin+probiotic treatment ameliorated minor abnormalities in ovaries as compared with the cisplatin group fishes as shown in (Fig. 2). Histopathological examination of the ovarian tissue reveals that cisplatin toxicities caused abnormal structural changes in the tissue. Considering histopathological observation, an observable difference has been observed between cisplatin and probiotic-treated tissues. Therefore, it may be suggested that the consumption of probiotics along with cisplatin might inhibit damage and reduce side effects. An intracellular ROS scavenging activity has been investigated using the quinol and hydroquinone approach as represented in Fig. 3. As from the image, the ROS scavenging activity has been recorded at 79.7% and 67.4% in the intestine and ovaries, respectively, upon treatment with a probiotic. Similarly, after treatment of cisplatin, approximately 96.4% and 84.3% of ROS scavenging activities have been notified in the intestine and ovaries, respectively. However, the scavenging activity has been significantly enhanced in both organs [intestine (78.1%)+ovaries (72.7%)] after treatment with the probiotic+cisplatin as compared to the neat probiotic. Based on these investigations, probiotic+drug combination could be useful for the ROS radical scavenging applications in real samples. The production of quinol-hydroquinone adducts in the presence of intracellular ROS. When quinol was treated with zebrafish cells by intercellular ROS species, the brown color

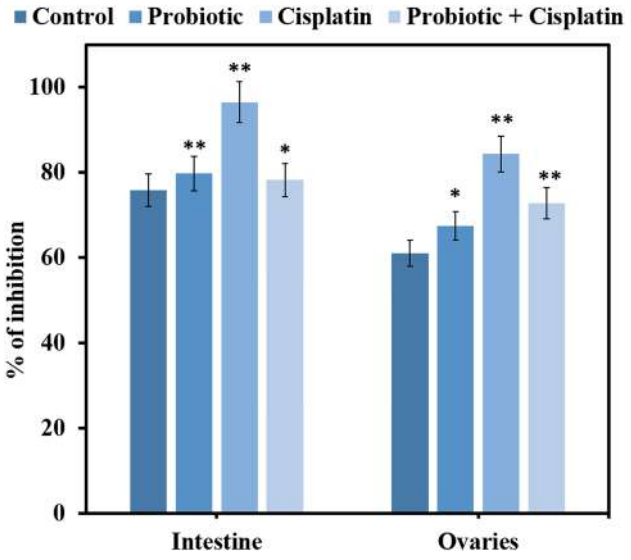

Fig. 3 Intracellular ROS scavenging activities in the intestine and ovaries after treatment. When quinol is treated with cells, reactive oxygen species (ROS) such as superoxide anion, peroxide, and hydroxyl radicals can be assessed utilizing the quinol oxidation followed by the formation of a matching adduct method in the presence of intracellular ROS. Following probiotic treatment, ROS scavenging activity was found to be 79.7% in the intestine and 67.4% in the ovaries. Similarly, following cisplatin treatment, approximately 96.4% and 84.3% of ROS scavenging capabilities were detected in the intestine and ovaries, respectively. However, when compared to the neat probiotic, the scavenging activity in both organs (intestine (78.1%)+ovaries (72.7%) was dramatically increased after treatment with the probiotic+cisplatin. (*) represents that they are significantly different ($P < 0.05$) from each other according to a one-way analysis of variance (ANOVA). (**) represents that they are significantly different ($P < 0.01$) from each other according to a one-way analysis of variance (ANOVA)

quinol-hydroquinone adduct was generated. The treatment of ROS species from zebrafish tissues caused the colourless quinol to produce an adduct with a brown hue called quinol-hydroquinone.

It is found that there was a statistically significant rise in the levels of lipid peroxidation, glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase in the cisplatin group compared to the control group in both the intestine and the ovaries. Administration of probiotics along with cisplatin significantly reduced this damage when compared to the control group. Histopathological analyses showed that the cisplatin group had much more damage than the control group and that probiotic+cisplatin treatment significantly restored these damages. It is evident from the results that the toxicity of cisplatin caused to increase in total lipid peroxidation activity in the intestine and was followed by ovaries tissues represented in Fig. 4i. ROS is capable of damaging molecules of biochemical classes including nucleic acids and amino acids. Exposure of reactive oxygen to proteins produces denaturation, loss of function, cross-linking, aggregation, and fragmentation of connective tissues. The most destructive effect is the induction of lipid peroxidation. The cell membrane which is composed of polyunsaturated fatty acids is a number one goal for reactive oxygen molecules, attacks leading to cell membrane damage. Probiotics are powerful antioxidants that protect cells from oxidative stress caused by reactive oxygen species, such as lipid peroxidation and DNA damage.

GPx (Glutathione peroxidase) is found in the cytoplasm of the cell and performs an important role in the detoxification of hydrogen peroxide. In the present study, a decrease in the activity of GPx was observed in the control group and probiotic group when compared with cisplatin-treated groups as shown in Fig. 4ii. Glutathione reductase (GR) is an antioxidant that protects cellular proteins against reactive oxygen species. Glutathione reductase is almost the same in the control, probiotic, and cisplatin+probiotic-treated groups except in the cisplatin-treated group as shown in Fig. 4iii. Many enzymes can neutralize hydrogen peroxide, these enzymes include catalase and glutathione peroxidase. Catalase is one of the most important antioxidants and a key enzyme that uses hydrogen peroxide, as its substrate. This enzyme is responsible for the neutralization of H_2O_2 into water and oxygen. Catalase activity is almost the same in the control, probiotic, and cisplatin+probiotic-treated groups except in the cisplatin-treated group as represented in Fig. 5i. SOD is a cellular enzyme that aids in the breakdown of potentially damaging oxygen molecules. This may help to prevent tissue injury. The presence of the enzymes catalase and SOD allows for the neutralization of harmful forms of oxygen. The enzyme acts as a good therapeutic active substance against reactive oxygen species-mediated diseases. It removes the superoxide anion by converting it to hydrogen peroxide, thus diminishing the toxic effect caused by this radical. An increase in the percentage inhibition of superoxide is seen in the cisplatin-treated group as shown in Fig. 5ii. This study examined the effectiveness of probiotics in preventing toxicities in zebrafish with oxidative ovarian damage brought on by cisplatin. The results of the investigation showed that cisplatin significantly increased oxidative stress in zebrafish ovarian tissues. Furthermore, compared to the other groups, the rate of toxicity was much higher in the cisplatin group, where oxidative stress was substantial.

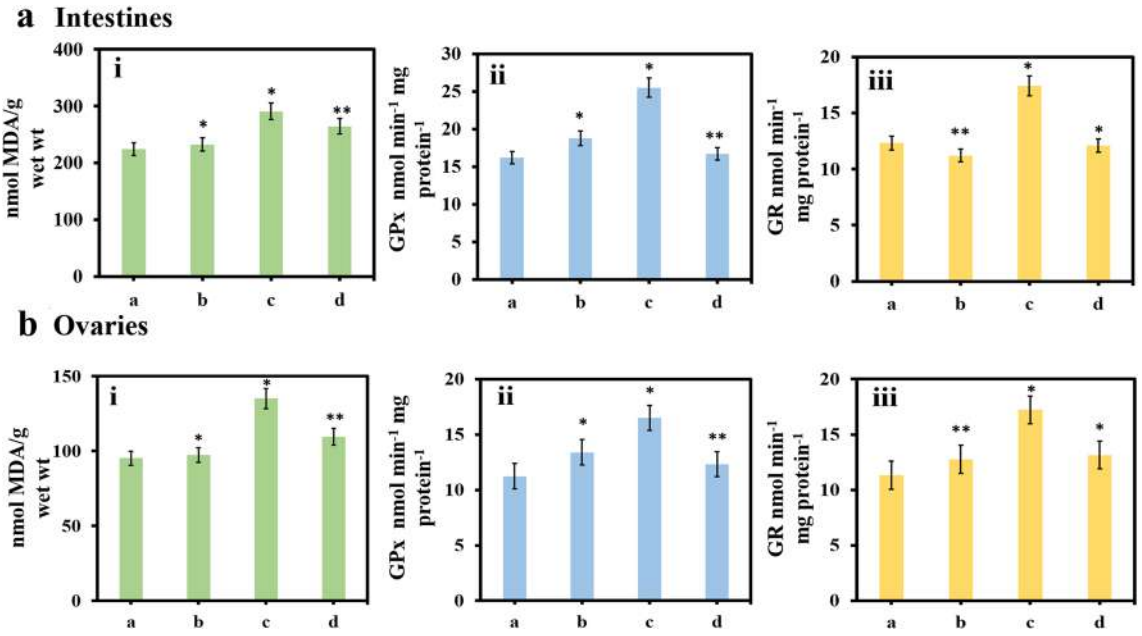

Fig. 4 Levels of (i) Lipid peroxidase (ii) Glutathione peroxidase (iii) Glutathione reductase in **A** Intestine **B** ovaries of (a) control (b) probiotic (c) cisplatin (d) probiotic+cisplatin. MDA (Malondialdehyde) was evaluated in tissue homogenates based on the reaction with thiobarbituric acid (TBA) to form a pink color complex, MDA produced was determined with the absorbance coefficient of the MDA-TBA complex at 550 nm using 1, 1, 3,3-tetraethoxypropane (TMP) as the standard. By catalyzing the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) for the consumption of NADPH, the catalytic activity of glutathione peroxidase and glutathione reductase was evaluated spectrophotometrically at 340 nm. The toxicity of Cisplatin caused an increase in total Lipid peroxidation activity in the Intestine, which was followed by the ovaries tissues. Decreased GPx activity was observed in the control and probiotic groups when compared to the cisplatin-treated groups in this study. Glutathione reductase is almost the same in the control, probiotic, and Cisplatin+Probiotic-treated groups except in the cisplatin-treated group. (*) represents that they are significantly different ($P<0.05$) from each other according to a one-way analysis of variance (ANOVA). (**) represents that they are significantly different ($P<0.01$) from each other according to a one-way analysis of variance (ANOVA)

Probiotics have more ability in removing free radicals. In addition to the antioxidant status, further evidence from histopathological studies clearly emphasizes the therapeutic application of probiotics. Thus cisplatin-induced oxidative stress was best prevented by the addition of probiotics. This study sheds light on the mechanisms underlying cisplatin-induced toxicity, as well as evidence for the potential utility of Probiotics in the safer administration of cisplatin.

Since there is evidence that chemotherapy drugs can also induce somatic cell damage, which indirectly results in oocyte mortality, the ovaries are crucial in evaluating the toxicity of cisplatin. It is also likely that chemotherapy drugs are directly targeting the oocyte or the somatic cells. The oocyte depends on these follicular somatic cells for survival, thus if they are destroyed, the oocyte may also perish. There were not many changes in intestinal tissue when zebrafish were treated with probiotic+cisplatin. Further, the presence of cisplatin resulted in damage to the intestines and ovaries, indicating its toxicity. There have been observed behavioral changes, such as irregular swimming, restlessness, and surface gasping, which could be a result of the toxic compounds' avoidance reaction. The harmful effects of the cisplatin on the exposed fish may be causing them to gulp more oxygen and move their opercula more frequently to obtain more oxygen to meet their higher energy demands. When compared to cisplatin treatment alone, the administration of probiotics along with cisplatin had a greater beneficial effect and reduced ROS generation. According to a current study [20], probiotics and other functional foods may aid in the fight against cancer and increase the potency of anticancer drugs. Additional probiotic-based drugs should be developed, and further in-depth research into these drugs and their mechanisms is needed.

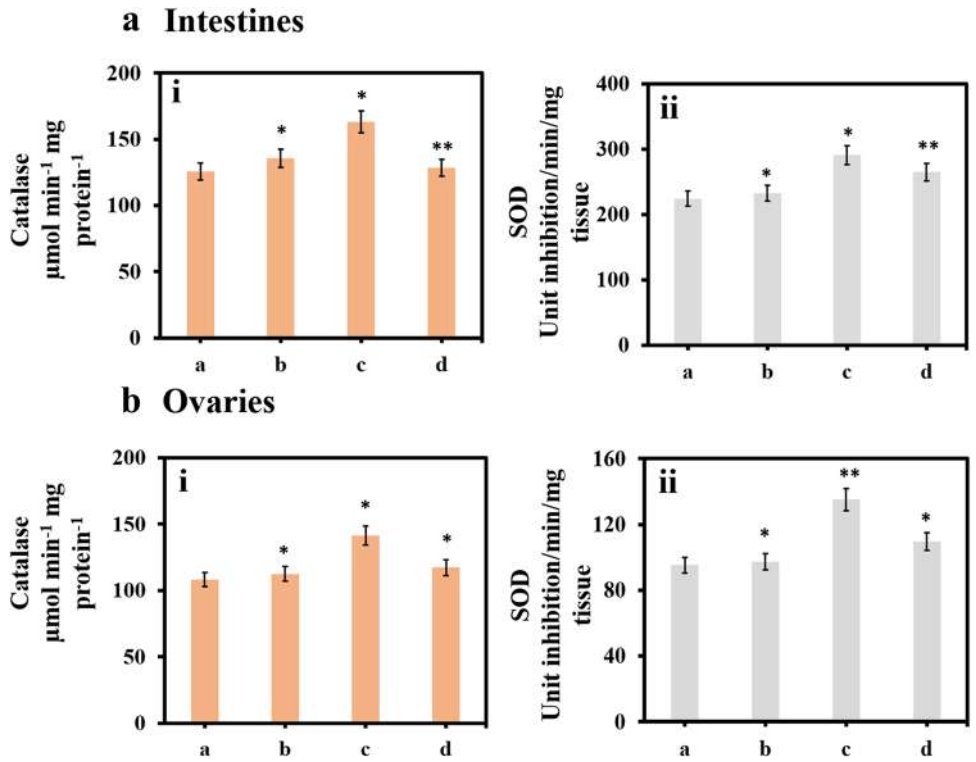

Fig. 5 Levels of (i) Catalase activity (ii) Superoxide dismutase activity in **A** Intestine **B** ovaries of (a) control (b) probiotic (c) cisplatin (d) probiotic+cisplatin. A spectrophotometric assessment of the breakdown of H2O2 at 240 nm was used to ascertain the catalytic activity of catalase. The modified NADH-phenazine methosulphate-nitro blue tetrazolium formazan inhibitory reaction technique was used to measure superoxide dismutase activity in tissue homogenates. Catalase activity is nearly identical in the control, probiotic, and Cisplatin+Probiotic-treated groups except in the cisplatin-treated group. An increase in the percentage inhibition of superoxide is seen in the cisplatin-treated group is seen. (*) represents that they are significantly different ($P < 0.05$) from each other according to a one-way analysis of variance (ANOVA). (**) represents that they are significantly different ($P < 0.01$) from each other according to a one-way analysis of variance (ANOVA)

Conclusion

This current evidence highlights the potential of probiotics in reducing cisplatin-induced toxicity. Overall, the findings suggest that probiotics can improve zebrafish health by modulating antioxidative enzymes and cisplatin-induced tissue damage. It is worth mentioning that probiotics were used as an adjuvant in the chemotherapy of different tumors (lung, gastrointestinal tract, testis, prostate, cervix, and ovarian cancer) improving the rates of tumor regression as well as increasing survival and the quality of life of human cancer patients. As a result, future cancer treatments will combine probiotics and their products with immunotherapeutic and traditional methods to precisely target cancer cells. It is important to keep studying the anti-cancer properties and mechanisms of action of probiotic strains, especially during treatment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-023-03203-5.

Acknowledgements The authors wish to thank Dr. Subramanyam Dasari, Indiana University and Dr. Mallikarjuna Nimgampalle, IISER Tirupati, India for their help in revising this manuscript. We are also thankful to Dr. Buddolla's Institute of Life Sciences for their technical support.

Author Contributions KK: Methodology, Data curation, Formal analysis, Writing – original draft. ASR: Data curation, Investigation, Formal analysis. JSN: Data curation, Investigation, Formal analysis, Supervision. BV: Conceptualization, Investigation, Supervision, Validation, Funding acquisition, Project administration, Writing—review & editing.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval All experiments have been conducted as per the guidelines of the Institutional Animal Ethics Committee, Dr. Buddolla's Institute of Life Sciences, Tirupati, India (Registration No. 2172/PO/Re/S/2022/CPCSEA dated 9th June 2022

References

1. Górska A, Przystupski D, Niemczura MJ, Kulbacka J (2019) Probiotic bacteria: a promising tool in cancer prevention and therapy. Curr Microbiol 76:939–949
2. Dasari S, Tchounwou PB (2014) Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 740:364–378
3. Karasawa T, Steyger PS (2015) An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. Toxicol Lett 237(3):219–227
4. Johnstone TC, Suntharalingam K, Lippard SJ (2016) The next generation of platinum drugs: targeted Pt (II) agents, nanoparticle delivery, and Pt (IV) prodrugs. Chem Rev 116:3436–3486
5. Ozen M, Dinleyici EC (2015) The history of probiotics: the untold story. Benef Microbes 6:159–165
6. Marullo R, Werner E, Degtyareva N, Moore B, Altavilla G, Ramalingam SS, Doetsch PW (2013) Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. PLoS ONE 8(11):e81162
7. Kandati K, Belagal P, Nannepaga JS, Buddolla V (2022) Role of probiotics in the management of cervical cancer: an update. Clin Nutr ESPEN. https://doi.org/10.1016/j.clnesp.2022.02.017
8. Sun B, Liu M, Tang L, Hu C, Huang Z, Zhou X, Chen L (2021) Probiotic supplementation mitigates the developmental toxicity of perfluorobutanesulfonate in zebrafish larvae. Sci Total Environ 799:149458
9. Waziri A, Bharti C, Aslam M, Jamil P, Mirza M, Javed MN, Alam MS (2022) Probiotics for the chemoprotective role against the toxic effect of cancer chemotherapy. Anti-Cancer Agents Med Chem 22(4):654–667
10. Azizpour Maghvan M, Jafari PJ, Anushiravani AA (2022) Investigating the influence of probiotic bacteria on anemia and white blood cells count in cancer patients undergoing chemotherapy and radiation therapy. Multidiscip Cancer Investig 6(3):1–6
11. Jakubauskas M, Jakubauskiene L, Leber B, Horvath A, Strupas K, Stiegler P, Schemmer P (2022) Probiotic supplementation suppresses tumor growth in an experimental colorectal cancer liver metastasis model. Int J Mol Sci 23(14):7674
12. Alam Z, Shang X, Effat K, Kanwal F, He X, Li Y, Zhang Y (2022) The potential role of prebiotics, probiotics, and synbiotics in adjuvant cancer therapy especially colorectal cancer. J Food Biochem. https://doi.org/10.1111/jfbc.14302
13. Ruch RJ, Crist KA, Klaunig JE (1989) Effects of culture duration on hydrogen peroxide-induced hepatocyte toxicity. Toxicol Appl Pharmacol 100(3):451–464
14. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2):351–358
15. Carlberg IN, Mannervik BE (1975) Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem 250(14):5475–5480
16. Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophysics.
17. Aebi H (1984) Catalase in vitro. Meth Enzymol 105:121–126
18. Jia M, Wang Y, Wang D, Teng M, Yan J, Yan S, Meng Z, Li R, Zhou Z, Zhu W (2019) The effects of hexaconazole and epoxiconazole enantiomers on metabolic profile following exposure to zebrafish (*Danio rerio*) as well as the histopathological changes. Chemosphere 226:520–533
19. Yan F, Polk DB (2011) Probiotics and immune health. Curr Opin Gastroenterol 27(6):496
20. Molska M, Reguła J (2019) Potential mechanisms of probiotics action in the prevention and treatment of colorectal cancer. Nutrients 11(10):2453

Clinical Nutrition ESPEN 48 (2022) 5–16

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Narrative Review

Role of probiotics in the management of cervical cancer: An update

Kusuma Kandati [a, c, 1], Praveen Belagal [b, 1], John Sushma Nannepaga [a, *], Buddolla Viswanath [c, **]

[a] Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, 517 502, India
[b] DST Purse Centre, Sri Venkateswara University, Tirupati, Andhra Pradesh, India
[c] Dr. Buddolla's Institute of Life Sciences, Tirupati, 517502, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 23 May 2021
Accepted 16 February 2022

Keywords:
Cervical cancer
Human papillomavirus
Probiotics
Lactobacillus
Treatment

SUMMARY

Studies have extensively investigated a variety of health benefits associated with probiotic supplements, which often contain live microorganisms. The effect of probiotic supplements on cancer prevention and on chemotherapy effectiveness and toxicity are major areas that researchers have focused on. Recently, several researchers have concentrated on assessing the efficacy of probiotics in the treatment of cervical cancer, a leading malignancy in gynecology worldwide, especially in developing countries. To date, numerous clinical studies have demonstrated the efficacy of probiotics in preventing cervical cancer, but their dosages, bacterial strains, and duration of therapy are somewhat inconsistent. In this review, we have systematically updated the role of probiotics in cervical cancer management.

1. Introduction

Cervical cancer is one of the most common cancers affecting women's health worldwide, with a comparatively rare occurrence in developed countries, but about 80% of cases occur in developing or less developed countries [1]. It is usually asymptomatic during the early stages, but subsequent signs include discomfort during sexual intercourse, pelvic pain, irregular vaginal bleeding, tiredness, leg swelling, etc. [2]. Moreover, cervical cancer is considered the fourth most prevalent malignancy among women worldwide; most incidences occur between the ages of 35 and 44 during midlife, with the average diagnostic age being 50 [3]. Persistent infections of human papillomavirus (HPVs), which are the driving force behind global cervical cancers, are attributed in almost all instances [4] and other risk factors include chewing tobacco and multiple sexual partners [5].

HPVs are incredibly complex oncogenic viruses that comprise more than 200 genotypes which are known to infect humans and animals. They are small, non-enveloped DNA viruses that are normal in nature and form a wide family of viruses that are both benign and highly carcinogenic [6]. Based on their variations in pathogenicity, HPV types can be broadly divided into high- and low-risk categories [7]. Benign lesions such as lower-grade squamous intraepithelial lesions (LSIL) and genital warts are caused by low-risk types such as HPV6, HPV11, and HPV30, while high-risk types such as HPV16, HPV18, and HPV58 predominantly cause higher-grade squamous intraepithelial lesions (HSIL) that lead to cervical cancer [7,8]. In the cervix, by minor abrasions in the tissue, initial infection is thought to occur in the epithelial basal cells [9]. Once HPV has reached the target cells, it can remain latent in the genome-integrated nucleus or follow replication, terminating the synthesis and release from the superficial cells of infective viral particles [10].

HPV infections elicit immune responses, which clear most HPV viruses. Persistent infections greatly increase the risk for carcinogenesis. Understanding the mechanisms of HPV-caused cancer could be helpful for the prevention and treatment of the disease [11]. As key care strategies for cervical cancer, chemotherapy, radiotherapy, and surgery have been considered. These therapies, however, have been shown to increase toxicity and side effects, which can have a detrimental impact on the lives of women with this cancer [12,13]. While most HPV infections can be eradicated by the immune system, only a small percentage of women's immune systems fail to clear the infected HPV, causing malignancy of the

* Corresponding author.
** Corresponding author.
E-mail addresses: johnsushma@gmail.com (J.S. Nannepaga), buddolla@gmail.com (B. Viswanath).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.clnesp.2022.02.017

uterus cervix [14]. A variety of beneficial health maintenance microbes are hosted by the human body where most of these microbes are found in the gut. The role of probiotics in improving gut functioning is depicted in Fig. 1. Any variation in the proportion of these microbes contributes to dysbiosis and leads to diseases such as cancer, metabolic and neurological diseases [15]. Prebiotics and probiotics work together to enhance the immune system by encouraging the growth of beneficial microorganisms [16]. HPVs infect host epithelial cells (keratinocytes) by interacting with different cell surface receptors such as integrin and heparan sulfate proteoglycans (HSPGs). The HPVs replicate themselves using the host cell replication apparatus to express E6/E7/E5 oncoproteins to immortalize the infected cells not only by inhibiting tumour suppressors p53 and Rb and decreasing apoptosis, but also importantly by activating the PI3K/Akt/mTOR signalling pathway. All these processes enhance cell proliferation leading to the carcinogenesis.

The dominant genus seen in the vaginal tract was *Lactobacillus,* with acidic secretions such as lactic acid, H_2O_2 etc., maintaining a pH range from 3.5 to 4.5 [17]. This acidic environment helps to prevent pathogens by developing cytokines that suppress inflammation and also stimulate the host [18]. Studies have shown that the physiological anatomy of the genitals, endocrine function and immune system of the human body may strongly influence the vaginal microbiota [17,19]. The ability to inhibit the proliferation of several forms of cancer cells has been shown by *Lactobacilli*, and recent epidemiological and experimental research has shown a clear correlation between increased probiotic intake and reduced cancer progression [20–22]. Interestingly, studies have shown that by influencing HPV clearance, by inducing cancer cell apoptosis and other anticancer activities, probiotics play an important role in combating cancer [17,23]. Many studies in the last few decades have added to our knowledge of probiotics' antioxidant activity. As a result, the objectives of this paper was to investigate into the potential health benefits of probiotics in the treatment of cervical cancer as well as their antioxidant activities.

2. Role of probiotics in olden day's health management and now how we use

Probiotics are not an invention but existed in our traditional foods such as beverages, salty fishes, yogurt, different types of cheeses and so on since olden times [24]. Such food structures contain different types of useful bacteria. It might be that the first real use of food containing Probiotics was fermented milk [25].

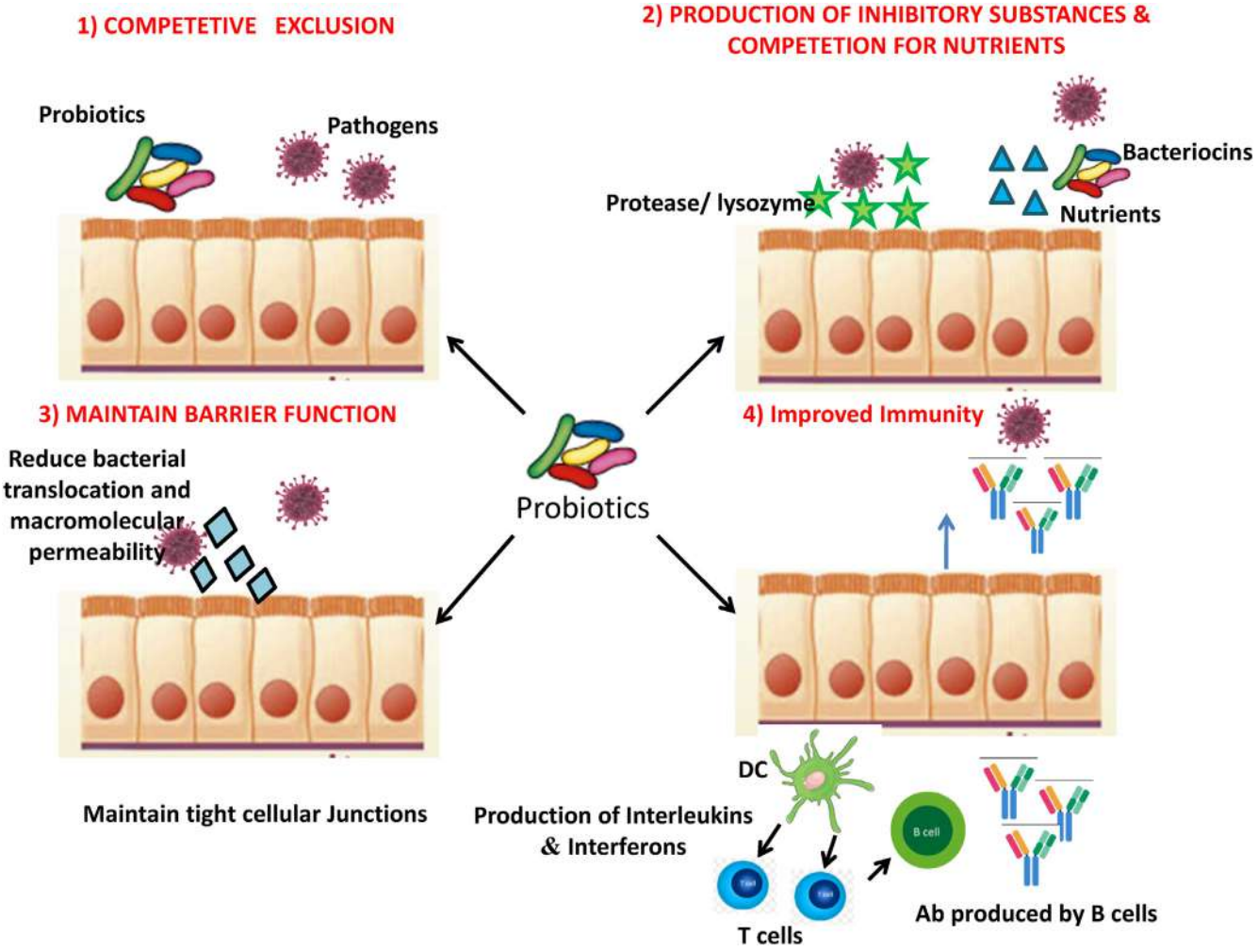

Fig. 1. Role of probiotics in the amelioration of gut functions. (1) The probiotics could protect from pathogenic microbes in a diversified manner. (2) The probiotics can secrete the antimicrobial compounds and metabolites such as lactic acid, enzymes, and bacteriocins which can alter the pH that ultimately can inhibit the growth of pathogens. (3) The probiotics maintain the gut mucosal barriers, i.e., the chemical barriers such as antimicrobial peptides (AMPs), which can inhibit the invading microorganisms, and the physical barriers including cellular junction and the mucus layer that repel the invading microorganisms. (4) The alliance of microbiota with the immune system allows the maintenance of regulatory function involved in the conservation of tolerance to safer antigens and induction of protection to the pathogens.

Humans learned that fermented milk has a good taste. Later they learned how to convert it into cheese, yogurt and so on [24,26,27]. Ilya Ilyich Metchnikoff, the Nobel Prize winner in Medicine in 1908, at the Pasteur Institute was the first who spotted the effect of what is called now Probiotic. He linked the health and longevity to the ingestion of bacteria present in yogurt [27]. Probiotics as a term was first used by Lilly & Stillwell in1965 to describe the 'substances secreted by one microorganism that stimulate the growth of another'. Parker [28] proposed that Probiotics are 'organisms and substances which contribute to intestinal microbial balance'. Probiotics confer health benefits on the host when given in sufficient quantities, including food digestion, the development of beneficial products to kill harmful microbes, enhance the functions of missing digestive enzymes (due to missed or defective genes), and to preserve the digestive system's pH [20,29]. After a long history of safe use of probiotics in fermented dairy products and an increased recognition of their beneficial effects on human health [30]. Consumption of probiotics, in the form of food or supplements, is a simple and feasible way of manipulating the composition of microbiota and a number of recent studies reflect a promising approach to prevent the onset and progression of cancer [10]. Probiotics have become a typical ingredient in many traditional foods and formulations hence the Food and Drug Administration (FDA) endorse probiotics for their virtually null safety issues [31]. Why must we use Probiotics? During our lives, we are exposed to different types of microbes, which are unsuitable for our health. Antibiotic treatment could destroy our useful microflora. In such cases, Probiotics should be used to regenerate our microflora [20].

The bacteria must be able to live in the acidic state of the stomach and bile acid at the start of the intestine to act as a probiotic, and several studies have shown that probiotic strains such as *Lactobacillus* and *Bifidobacterium* cause therapeutic and anti-colon cancer effects in tumor-bearing animal models against preneoplastic lesions [32]. However, the mechanisms by which cancer may be suppressed by probiotics are not completely understood [2,33]. Extensive research using human cancer cells has shown that in a large range of cancer cells, including colon, stomach, breast, cervix, and myeloid leukemia cells, probiotics have antiproliferative or proapoptotic activities [34–36]. Many factors in our lives disrupt our beneficial microflora; in these situations, exo-sources should be utilized. Such helpful bacteria can be found in exo-sources. **The benefits of probiotic bacteria**.

i. Probiotics are useful and friendly microbes.
ii. They are able to compete with the bad microbes and colonize our digestive system.
iii. They are able to ferment our food to simpler byproducts and could promote our health by many different mechanisms.
iv. Their amount could be deteriorated due to many factors, such as incorrect diet, alcohol, age and so on. This is why they should be taken through our regular diet.
v. In particular cases such as after antibiotic treatments, where they are expected to be affected severely, they should be taken orally in considerable amounts or with food.
vi. Probiotics promote health while they:
vii. Remove the side effect of the pathogens or the harmful microbes.
viii. Supply the body with useful byproducts.
ix. Reduce the jobs of our digestive system.
x. Reduce the effect of the first attack of harmful compounds, instead of our cells, by their biofilm, which protects our digestive system.
xi. Reduce the amount of food needed by our bodies due to the correct digestion and metabolism of any amount of food.
xii. Probiotics in some cases could complement the deficiency in our genetic materials by helping us to borrow the products of their genes (such as in case of the lactose fermentation deficiency).

3. HPV induced cervical cancer

The vast majority of HPV infections are transitory and become undetectable in 12–24 months [37–41]. However, in some women whose infections continue to persist, the risk of developing precancerous conditions is significant. Cervical cancerogenesis can be defined as the complex mechanism of uncontrolled cellular division that can involve HPV gene integration together with other cellular changes and epigenetic factors. As the HPV infection occurs, the DNA can undergo mutations under the cellular and other environmental conditions leading to viral DNA integration and operation with the host DNA synthesis machinery. As a result, virus can escape cellular and immune defense mechanisms while promoting cell proliferation and inhibiting cellular apoptotic mechanisms [42]. Fig. 2 depicts the molecular mechanism by which HPV causes oncogenesis by inactivating tumour suppressor genes.

HPV 16 and 18 are high-risk types known to significantly increase the risk of cervical, vaginal, and vulvar cancer in women. Oncogenic potential of HPV16 depends on the regulation of viral transcriptional factors. At the initiation of viral infection, the HPV16 genome can be presented as unintegrated small DNA molecule also called episome and results in benign and precancerous lesions of the cervix. However, HPV16 can integrate its genome into the host genome, which in turn can lead to the development of cervical carcinoma and cervical intraepithelial neoplasia grade III [43]. Viral genome integration in combination with dysregulation of the E2 protein, which is a repressor of the oncoprotein, contributes towards the carcinogenic process. These events cause overexpression of E6 and E7 proteins that eventually contribute to viral carcinogenesis by altering cellular apoptotic mechanism [44]. Overexpression of E6 and E7 alone is insufficient to contribute to the cancerogenesis as other genetic and epigenetic factors also need to be established [42].

The objective of this comprehensive study is to look at how probiotics can help with cervical cancer treatment. Furthermore, when probiotics are employed in a treatment strategy, it is important to consider their safety. However, more research is needed to determine whether probiotics are useful in the treatment of cancer in clinical settings. We also reviewed the function of probiotics in the management of health in the past, as well as how we utilize and benefit from probiotics in the treatment of cervical cancer.

4. Outline for the beneficial role of probiotics on cervical cancer treatment management

Research data indicates that the regular use of probiotics and prescription drugs contributes to better treatment outcomes [45–47]. Moreover, probiotics have been shown to provide a better screening tool for cervical cancer patients when paired with anti-infective medications [13]. There are thousands of bacterial species that could provide and sustain health in the human body and among these species; about nine percent of microbial species have been found in the urogenital tract [48]. Therefore, there could be a harmony in the urogenital tract between the immune system and the host microbiota, and disruptions in this harmony can lead to cervical cancer by allowing infectious agents to proliferate in this area [49,50].

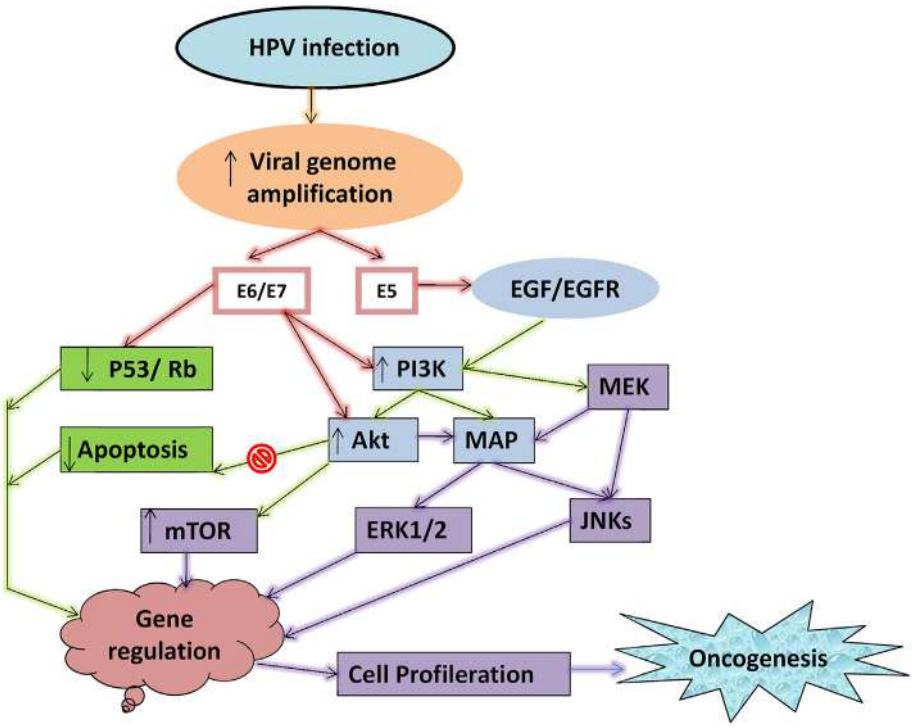

Fig. 2. Molecular mechanism of HPV induced oncogenesis through the inactivation of tumor suppressor genes.

Several studies have concentrated on the possible roles of probiotics for cervical cancer prevention, treatment and recovery [51–53]. The effect on genital high-risk human papilloma virus (HR-HPV) infections of probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 has decreased rates of slightly irregular and unsatisfactory cervical smears [54]. A total of 121 women with genital HR-HPV infection were included in this study [54], with 62 in the study group and 59 in the control group. The HR-HPV clearance rate did not differ significantly between the two groups, according to the findings (58.1% vs. 54.2%). A lower initial viral load, however, was the only factor predicting HR-HPV clearance. Their findings indicated that there was a mild abnormal initial cervical smear in twenty-two women, whereas nine of them had an unsatisfactory smear. Both mildly abnormal cervical smear and unsatisfactory smear rates decreased significantly in the study group compared to the control group at 6 months of follow-up [54].

5. Probiotics inhibits/suppress the cervical cancer through apoptosis

The apoptosis signaling pathways can be activated by lactic acid bacteria (LAB) through the extrinsic and intrinsic pathways. The extrinsic pathway engages Fas/tumor necrosis factor receptors or other factors to induce caspase related pathway. The intrinsic pathway requires mitochondrial localization and activation of Bax and Bak that can be prevented by anti-apoptotic Bcl-2 family proteins or pharmacologic inhibitors. LAB enhanced the apoptosis induction capacity of 5-fluorouracil (5-FU) and induced the activation of autophagic cell death promoted directly by the induction of Beclin-1 and GRP78, as well as indirectly through the induction of Bcl-2 and Bak. LAB may act to prevent cancer via downregulating nuclear factor-kappaB (NF-κB)-dependent gene products which regulate cell proliferation (Cox-2, cyclin D1) and survival (Bcl-2, Bcl-xL).

6. Probiotics can help prevent cervical cancer

Reported mechanisms developed in cancer prevention and probiotic treatment to date, are schematically presented in Fig. 3, which includes.

(i) Gut microbiota modulation;
(ii) Enhancement of the function of the gut barrier;
(iii) Degradation of potential carcinogens and the ability to defend against DNA damage to the intestinal epithelium; and
(iv) Enhancement of the body's immune and inflammatory systems [7].

In order to identify and kill cells that may become cancerous, probiotics allow the immune system to operate at its best. Probiotics can alter the intestinal microbial environment, improve the function of the intestinal barrier, provide competitive adherence to the mucosal epithelium, generate antimicrobial substances, and modulate immune responses by improving the innate and adaptive immune response [33]. In a study involving 54 women, daily probiotic intake was found to increase HPV clearance for 6 months, which is known to be the primary cause of cervical cancer [51]. Differences between particular anatomic locations inside the vagina are likely to be found in the mucosal compartments. There have been attempts to analyze some vaginal compartments (cervix vs. proximal vagina vs. distal vagina), but other locations, including ectocervical and endocervical or microbiota associated with immature and mature cervical epithelium, remain poorly characterized [55]. The Molecular changes when cancer cells treated with *Lactobacillus* and other probiotic supernatants are described in Fig. 4. Due to the near proximity of these sites where contamination can occur during the sampling process, analysis of these studies is also difficult. It is difficult to describe “healthy” microbiota as population clustering seems to be a moving target with variability across the menstrual cycle of women as well as their reproductive

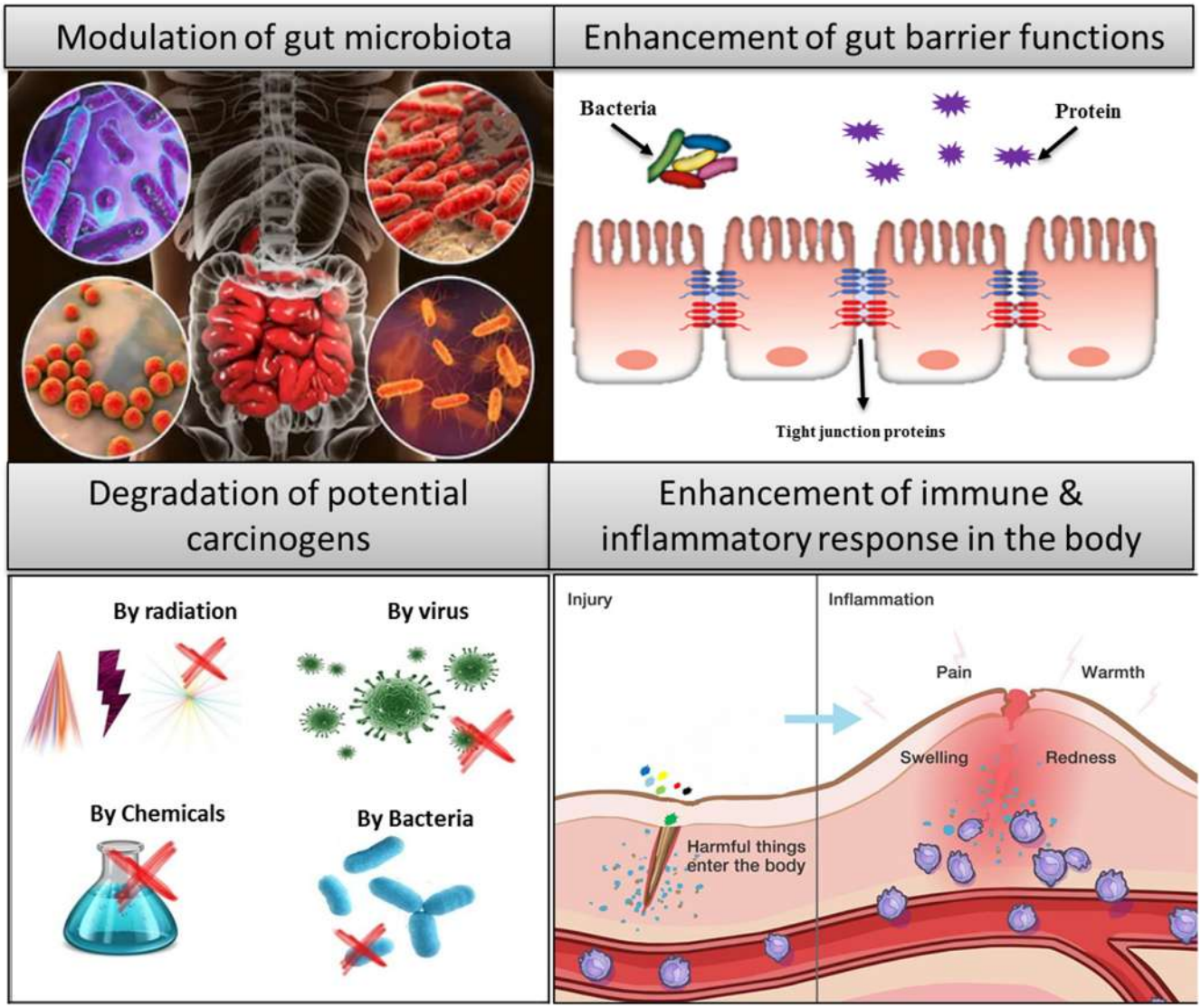

Fig. 3. Schematic representation of cervical cancer prevention with probiotics.

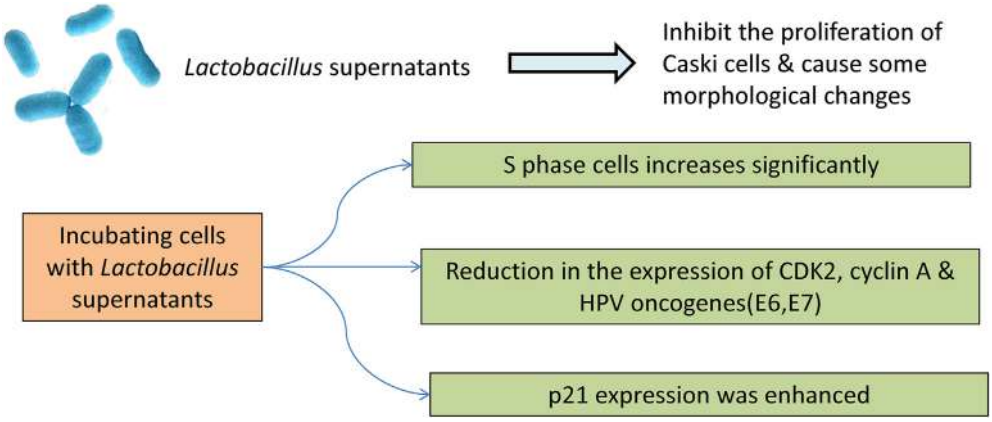

Fig. 4. Molecular changes when cancer cells treated with *Lactobacillus* and other probiotic supernatants.

age [55]. Recent findings have shown remarkable probiotic abilities, which may lead to the potential prevention or treatment of cervical cancer through.

- Apoptosis induction,
- Proliferation inhibition,
- Decreased inflammation, and
- Metastasis suppression [13].

6.1. Experimental findings

Recent experimental evidence has shown that *Lactobacillus* supernatants (LS), *Lactobacillus crispatus*, *Lactobacillus jensenii, and Lactobacillus gasseri*, inhibit the proliferation of CaSki cells (contain an integrated human papillomavirus type 16 genome) causing favorable morphological alterations (in a pH-independent manner) [22]. The number of S phase cells increased significantly by incubating cells with LS; meanwhile, G2/M phase cells decreased [22]. The proteins linked to p53 and pRB tumor suppressors and necessary for conversion to malignancy are encoded by the E6 and E7 genes [56]. Treatment with LS results in a decrease in the oncogenesis of CDK2, cyclin A, and HPV expression (E6 and E7). In addition, in LS-treated cells, p21 expression was enhanced [22]. *Lactobacillus plantarum*, isolated from vaginal secretions of young adult and adolescent women, have been shown to have probiotic characteristics and anti-cancer activities against the HeLa line of cervical cancer [57] mentioned in Table −1. Another HeLa cell line research revealed that human milk-isolated strains of Lactobacillus (*L.casei SR1, L.casei SR2, and L.paracasei SR4*)) have remarkable probiotic activities, findings show that supernatants of cell-free culture have anticancer activities, such as regulating BCL-2 down and up regulating apoptotic genes (caspase3, caspase8, caspase9, BAX, and BAD) [2]; Sungur [58] reported that human vagina-isolated *L. gasseri* strains, G10 and H15, inhibit the HeLa cells proliferation. It is observed that treating HeLa cells with supernatants of *L. rhamnosus* and *L. crispatus* reduces the expression of CASP3 gene as well as MMP2 and MMP9, which causes an inhibitory effect on metastasis [59]. Several studies have been performed, both in vitro and in vivo; suggesting that probiotics are successful in the prevention and treatment of cancer, but the actual effect of probiotics can only be tested in *vivo* studies [60]. The underlying mechanisms for their anti-cancer effects are flexible, including reducing the growth of microbiota involved in the development of mutagens and carcinogens, altering the metabolism of carcinogens, protecting DNA from oxide damage, and regulating the immune system [61]. Assays have been performed to evaluate sufficient doses to investigate the inhibitory effect of *lactobacilli* on in vitro HeLa and U14 cell migration capabilities. In addition, to examine the potential pathways corresponding to its antitumor effects, Western blot assays were performed. The results showed that live lactobacilli [1000:1 multiplicity of infection (MOI)] had significant inhibitory effects on cervical cancer cell migration ability. E-cadherin expressions in HeLa and U14 cells were significantly upregulated by *Lactobacilli* here. Results, on the other hand, showed that inactivated *Lactobacilli* in HeLa and U14 cells did not affect the levels of E-cadherin expression [62].

7. Combination of probiotics with other cervical cancer therapies

Cisplatin has been one of the most important chemotherapeutics in patients with advanced cervical cancer [12]. It is reported that cisplatin pro-apoptotic and antigrowth effects are enhanced by co-treatment with *Lactobacillus* bacteria in mouse models with cancer [64].

A study has suggested that the well-balanced intestinal microflora plays a protective role in the treatment of cancer [65]. Various platforms such as metagenomics, metatranscriptomics and culturomics, could be beneficial in differential microbial composition in health and disease. The data generated together can indicate which bacterial genera or species could be beneficial to patients [66]. Therefore, cancer treatment with microbiome or their products has the potential to treat tumors. However, it has also been implicated that the microbial agents could also suppress cancer prognosis via production of potentially oncogenic toxins and metabolites. Thus, future treatments would rely on the use of a combination of microbiome and its products with immune-therapeutics and more conventional approaches to target directly the malignant cells [66].

8. Molecular mechanism of probiotics as therapeutic strategy

Probiotics have demonstrated significant potential as therapeutic options for a variety of diseases, but the mechanisms responsible for these effects have not been fully elucidated yet [70]. Probiotics have been reported to play a therapeutic role by enhancing immunity, lowering cholesterol, improving lactose tolerance and preventing some types of cancers. The markets for probiotic products and supplements are growing worldwide because of various benefits [71] (see Table 1).

9. Impact of probiotics on diarrhea caused by chemotherapy

In people with cervical cancer, diarrhea is one of the most common and problematic side effects associated with chemotherapy or radiotherapy [72,73]. The prevalence of these adverse effects was as high as 50–80%. Extreme diarrhea triggered by treatment may contribute to loss of fluids and electrolytes and nutritional deficiencies and may adversely affect the quality of life [74]. In addition, owing to anticancer therapy, patients with neutropenia are more prone to diarrhea, which may also lead to dosage changes or discontinuation or delayed care [75]. In preventing or treating chemotherapy-or radiotherapy-induced diarrhea, probiotics can be successful [76]. Probiotics have positive effects on the frequency and incidence of diarrhea and the need for rescue treatment in patients undergoing radiotherapy (with or without chemotherapy) [77]. In the study, patients undergoing radiotherapy with or without chemotherapy were given placebo-related probiotics to avoid diarrhea [78]. Of these, certain patients who had already acquired grade 1 diarrhea at baseline were included in one research while another study discussed grade 2 or higher diarrhea prevention. The first study involved 63 patients undergoing pelvic radiotherapy concurrently with weekly cisplatin, investigating the comparison of live *Lactobacillus acidophilus + Bifidobacterium bifidum* (n = 32) with placebo (n = 31). In terms of individual characteristics or the pelvic radiotherapy protocol at baseline, researchers found no substantial variations between the two types. Four results of concern were recorded in the study: the percentage of participants with diarrhea, the severity of diarrhea, the proportion of participants needing rescue treatment and the adverse incidents [78]. Latest studies of the in vitro impact of probiotics on cervical cancer cells have been summarized in Table 2.

In another study, researchers assessed the effectiveness of a high-potency probiotic preparation in people with cancer for the prevention of radiotherapy-induced diarrhea [85]. This study included 490 participants who were randomly assigned to VSL # 3 (n = 245) or placebo (n = 245) treatment. VSL#3 contained *Lactobacillus casei, L. plantarum, L. acidophilus, Lactobacillus*

Table 1
Studies investigating probiotics effects on cervical cancer cells.

Probiotic	Cell line	Findings	References
L. crispatus, *L. jensenii*, and *L. gasseri*	Caski	Inhibition of the viability by regulation of HPV oncogenes and cell cycle-related genes	[22]
Vagina-isolated *L. plantarum*	HeLa	Suppression of proliferation and induction of apoptosis	[57]
Milk-isolated *L. casei* and *L. paracasei*	HeLa	Induction of apoptosis	[2]
Vagina-isolated *L. gasseri*	HeLa	Inflammation and proliferation were reduced and apoptosis was increased	[58]
L. rhamnosus and *L. crispatus*	HeLa	Proliferation and metastasis were suppressed	[59]
Bifidobacterium adolescentis SPM1005-A	SiHa	Suppression of E6 and E7 oncogenes	[63]

Table 2
Indirect impact of probiotics on cervical cancer cells.

Probiotic strain	Benefits	Reference
L. rhamnosus 573	Patients had less abdominal discomfort, with less hospital care and fewer chemo dose reductions.	[79]
L. acidophilus plus B. bifidum (Infloran®)	Reduction in incidence of diarrhea and better stool consistency	[73]
L. plantarum CGMCC 1258, *L. acidophilus* LA-11, *B. longum* BL-88	Significant improvement in the integrity of gut mucosal barrier and reduction in infections complications.	[80]
L. casei Shirota (LcS)	Significant evidence of cancer preventing particularly colorectal cancer.	[81]
L. casei ATCC 393	Significant in vivo anti-proliferative effects accompanied by apoptotic cell death in colon carcinoma cells.	[82]
L. acidophilus SNUL *B. longum* HY8001	Inhibit growth of tumor cell, produce anti-carcinogens and reduces cancer risks	[83]
L. paracasei IMPC2.	Anticancer activity	[84]

delbrueckii subsp. *bulgaricus*, *Bifidobacterium longum, Bifidobacterium breve*, *Bifidobacterium infantis,* and *Streptococcus salivarius* subsp *thermophilus*. One sachet of VSL#3 was given thrice a day, starting from the first day to the end of scheduled cycles of radiotherapy. Researchers found no major variations in baseline patient characteristics between the two classes. Five outcomes of concern were recorded in this study: proportion of participants with diarrhea, severity of diarrhea, drug rescue period, diarrhea-caused mortality, and adverse incidents [85]. Trialists evaluated these effects weekly until one month after completion of radiotherapy and the performance is very promising.

10. Probiotics as a physiological barrier to prevent the attachment of pathogens

Major Probiotic mechanisms of action include enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, and concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, and production of antimicroorganism substances and modulation of the immune system [70]. The intestinal barrier is a major defense mechanism used to maintain epithelial integrity and to protect the organism from the environment. Probiotics may promote mucus secretion as one mechanism to improve barrier function and exclusion of pathogens is depicted in Fig. 5.

The intestinal tract is a diverse microenvironment where more than 500 species of bacteria thrive. To protect itself from uncontrolled inflammatory responses, the epithelium has developed mechanisms to restrain bacterial growth, limit direct contact with the bacteria, and prevent bacterial dissemination into underlying tissue. Disruption of this barrier can lead to loss of immune tolerance to the microflora and an inappropriate inflammatory response, as is thought to occur in the inflammatory bowel diseases (IBD) ulcerative colitis and Crohn's disease [86–88]. The intestinal barrier defenses consist of the mucous layer, antimicrobial peptides, secretory IgA, and the epithelial junctional adhesion complex [89]. Probiotics are live microorganisms that confer benefit to the host and that have been suggested to ameliorate or prevent diseases including antibiotic-associated diarrhea, irritable bowel syndrome, and inflammatory bowel disease. Probiotics likely function through enhancement of barrier function, immunomodulation, and competitive adherence to the mucus and epithelium [90]. Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is important for the interaction between probiotic strains and the host [91–93]. Adhesion of probiotics to the intestinal mucosa is also important for modulation of the immune system [93–95] and antagonism against pathogens [80]. Thus, adhesion has been one of the main selection criteria for new probiotic strains [91,96–98] and has been related to certain beneficial effects of probiotics. Probiotics also cause qualitative alterations in intestinal mucins that prevent pathogen binding [99]. Probiotic strains can also induce the release of defensins from epithelial cells. These small peptides/proteins are active against bacteria, fungi and viruses. Moreover, these small peptides/proteins stabilize the gut barrier function.

11. Probiotics as potential antioxidants

In recent decades, many findings have shed new light on the understanding of the antioxidant capacity of probiotics. Oxidative stress defines a condition in which the prooxidant–antioxidant balance in the cell is disturbed, resulting in DNA hydroxylation, protein denaturation, lipid peroxidation, and apoptosis, ultimately compromising cells' viability. ROS mediated oxidative stress are known to play vital role in the development of chronic diseases such as cancer, diabetes, heart disease, stroke, Alzheimer's disease, rheumatoid arthritis, cataract and aging. Antioxidants are molecules which interact with free radicals generated in cells and terminate the chain reaction before damage is done to the vital molecules [100]. Consumption of probiotics alone or in food shows that strain-specific probiotics can present antioxidant activity and reduce damages caused by oxidation [101]. However, the oxidation-resistant ability of probiotics, especially the underling mechanisms, is not properly understood. Oxidative stress refers to elevated intracellular levels of oxygen radicals that cause damage to lipids, proteins, and DNA [102]. Reactive oxygen species (ROS), including

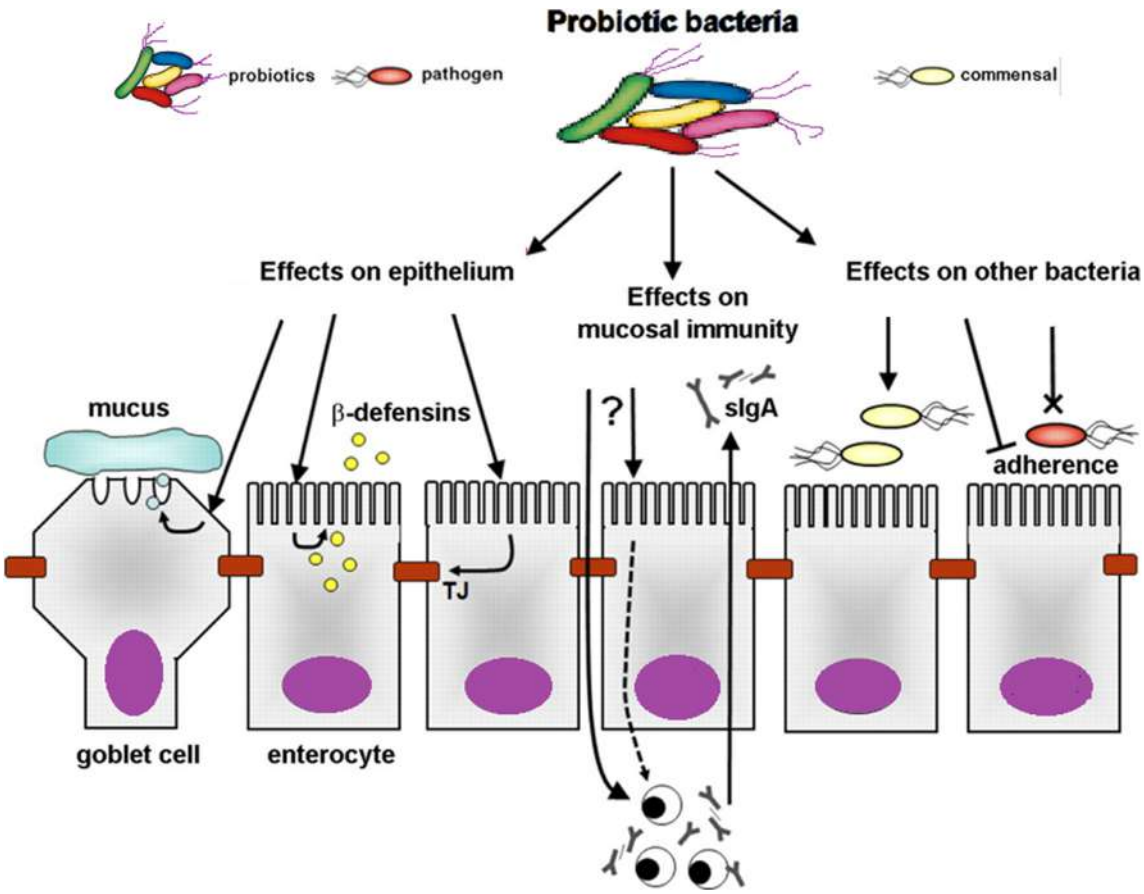

Fig. 5. Mechanisms by which probiotics directly or indirectly (perhaps via alterations in microflora) enhance the host barrier function. Effects of probiotic bacteria and on intestinal epithelial barrier function. Probiotics affect the epithelial barrier in numerous, diverse ways. This multifactorial approach to enhancing intestinal barrier function aids in developing and maintaining homeostasis. Depending on the strain of bacteria and the model used, probiotics target the epithelial barrier in the following 3 areas. *A*: direct effects on the epithelium. Probiotics can increase mucin expression and secretion by goblet cells, thereby limiting bacterial movement across the mucous layer. Augmentation of β-defensin expression and secretion into the mucus by epithelial cells can prevent the proliferation of commensals and pathogens, thus also contributing to barrier integrity. Finally, probiotics can enhance tight junction stability, which decreases epithelial permeability to pathogens and their products. *B*: effects on mucosal immunity. Probiotics can increase levels of IgA-producing cells in the lamina propria and promote secretory IgA (sIgA) secretion into the luminal mucous layer. These antibodies limit epithelial colonization by binding bacteria and their antigens, thus contributing to gut homeostasis. *C*: effects on other surrounding or infecting bacteria. Probiotics can alter the microbiota composition and/or gene expression, leading to indirect enhancement of the barrier through the commensal bacteria. Furthermore, some probiotics can directly kill or inhibit growth of pathogenic bacteria via expression of antimicrobial factors such as bacteriocins. Probiotics can also compete with pathogens or commensals for binding sites on mucins or epithelial cells, thereby preventing detrimental colonization and contributing to barrier function.

superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide, are one of the highly active oxygen free radicals. During evolution, most living organisms possess enzymatic defenses (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR)), non-enzymatic antioxidant defenses (glutathione, thioredoxin, Vitamin C, Vitamin E), and repair systems to protect them against oxidative stress [100]. However, these native antioxidant systems are generally not enough to prevent living organisms from oxidative damage. Antioxidant additives using substances that delay or prevent the oxidation of cellular substrates have demonstrated the capacity to protect the human body against oxidative damage. Recent studies have led to a renewed interest in probiotics, which are claimed to have health benefits. Evidence has showed that probiotic bacteria present significant antioxidant abilities both in vivo and in vitro [101,103–105]. Probiotic *Bifidobacterium* is also a very commonly used probiotic bacterium. It was able to promote antitumor immunity [106]. The culture supernatant, intact cells and intracellular cell-free extracts of *Bifidobacterium animalis* were found to scavenge hydroxyl radicals and superoxide anion in vitro while enhancing the antioxidase activities of mice in vivo [104]. It is revealed that LAB can resist ROS, including peroxide radicals [107], superoxide anions, and hydroxyl radicals [108]. The complete picture of the interaction between probiotics and antioxidant capacity should be further investigated and come into view in future.

12. Probiotics induces immune modulators

The immune system plays a pivotal role in the prevention and control of tumour initiation and progression [29]. The interaction of numerous elements of the immune system such as antigen presenting cells (APC), different subsets of T-cells, B-cells, natural killer cells and dendritic cells are usually activated during invasion or mutation [109,110]. Most importantly, modulation of the immune system is one of the most plausible mechanisms underlying the beneficial effects of probiotics on human health. Probiotics have been found to enhance the innate immunity and modulate pathogen-induced inflammation via toll-like receptor-regulated signaling pathways [111]. A recent study demonstrated how probiotics activated innate immunity to prime the adaptive immune responses. A probiotics mixture consisting of *L. acidophilus, L. casei, L. reuteri, Bifidobacterium bifidium*, and *Streptococcus thermophilus*

stimulated regulatory dendritic cells that express high levels of IL-10, TGF-β, COX-2, and indoleamine 2,3-dioxygenase, which in turn promoted the generation of $CD4^+Foxp3^+$ regulatory T cells (Tregs) from the $CD4^+CD25^-$ population and increased the suppressor activity of naturally occurring $CD4^+CD25^+$Tregs. In addition, this probiotic mixture induced both T-cell and B-cell hyporesponsiveness and downregulated T helper (Th) 1, Th2, and Th17 cytokines without inducing apoptosis. In-vivo studies revealed that this mixture suppressed 2,4,6-trinitrobenzenesulfonic acid-induced intestinal inflammation, which was associated with enrichment of $CD4^+Foxp3^+$ Tregs in the inflamed regions. Thus, probiotics that enhance the generation of regulatory dendritic cells to induce Tregs represent a potential therapeutic approach for inflammatory disorders [112]. The gut mucosal epithelium not only acts as a barrier to unwanted pathogenic organisms but represents a mechanism of safely and selectively tasting luminal contents of the gut, passing this information underneath the barrier to the immune cells/tissue of the (Gut Associated Lymphoid Tissue) GALT in the lamina propria and beyond in the mesenteric lymph nodes. This selective tasting of the contents of the GIT is the way in which the host tolerates that which is beneficial non-self (through the mechanism of immune tolerance/hyporesponsiveness) and mounts protective immune responses to that which is pathogenic non-self (through humoral and cell-mediated immune mechanisms). The process by which this antigenic information is passed to the underlying cells is crucial to this immune fate: tolerance/suppression *versus* activation. There are generally three mechanisms in which antigenic material is processed and presented to the underlying immune cells and that these mechanisms are controlled by three different types of antigen-presenting cells (APCs) [113].

13. Probiotics produces cytotoxic compounds/prodrugs against cancer cells

Probiotic bacteria have the ability to both increase and decrease the production of anti-inflammatory cytokines which play an important role in prevention of carcinogenesis. They are also capable of activating phagocytes in order to eliminate early-stage cancer cells. Probiotics have been gaining much attention due to their ability to modulate cancer cell's proliferation and apoptosis, investigated both in vitro and in vivo [60]. A specific mechanism associated with antitumor properties of probiotics remains unclear. Gut microbiota is engaged in a variety of pathways, which are considered to play a central role in that process. Primarily, probiotic bacteria play an essential role in the preservation of homeostasis, maintaining sustainable physicochemical conditions in the colon. Reduced pH caused inter alia by the excessive presence of bile acids in feces may be a direct cytotoxic factor affecting colonic epithelium leading to colon carcinogenesis [114,115]. Regarding their involvement in the modulation of pH and bile acid profile, probiotic bacteria such as *L. acidophilus* and *B. bifidum* have been demonstrated to be a promising tool in cancer prevention [114,116,117]. An early but controlled and comparative study on 223 patients carried out in 1993 showed that combination therapy including radiation and treatment with heat-killed *L. casei* strains (LC9018) and improved the induction of immune response mechanisms against cancer cells thereby enhancing tumor regression in patients with carcinoma of the uterine cervix [118].

14. Safety of the probiotics

Since numerous types of microbes are used as probiotics, safety is also intricately tied to the nature of the specific microbe being used. Safety assessment guidelines are needed to test the safety of probiotics, but taking into account the great diversity of microorganisms it is necessary to identify the specific risks associated with the different probiotics as well as the risk factors associated with the host and the possible interactions among the probiotic, host and food components [119]. Probiotics and their safety aspects have been important for evaluation of the therapeutic role of probiotics against infections and cancer. A properly assessed probiotic (diet) consumption/treatment plays a key role in the management of disease [120]. In addition to the long history of the safe use of lactic acid bacteria, oral administration of probiotics is well tolerated and has been proven to be safe in hundreds of clinical trials involving thousands of subjects [121]. Lactic acid bacteria in general have a good safety record. They are rarely involved in disease. Several probiotics have a long history of safe use. However, new probiotic strains will not have such a history and therefore need to be assessed for safety on a strain-by-strain basis. The scientific evidence on the health benefits of probiotics continues to increase. The benefits provided by probiotics are likely to outweigh any potential risks, but this risk/benefit ratio should be determined for each strain and target population. Therefore, specific work should be done for the assessment of potential probiotic pathogenicity, including the dose or duration of use. When administered, the probiotics are alive and capable of producing toxins or possible infection in the body. Probiotics are considered “generally recognized as safe,” and are widely used in the prevention and treatment of several diseases.

15. Conclusion

The role of probiotics in the treatment of cervical cancer is stated in this study. Because of its positive effect on the human body along with the avoidance and encouragement of the treatment of many diseases without side effects, auspicious impacts of probiotics are considered. Insights into cellular and molecular mechanisms such as immune responses, apoptosis, anti-oxidant and epigenetic mechanisms bring novel approaches for the development of probiotic-based therapeutics. Supplementation of dietary constituents is an emerging and safe strategy in cancer prevention. Luckily, but only in developed countries, there is a vaccine to combat cervical cancer as well as screening methodologies. The original avoidance strategy includes a healthy and balanced diet being continuously eaten. Apart from being treated, carcinogenic foods that can cause cancer cells must also be prevented. There is a great deal of evidence that the use of functional foods such as probiotics may play a significant role in combating cancer and in supporting anti-cancer treatments as well. Improving the understanding of the relationship between the immune system, microenvironment and probiotics will provide insights into the growth of probiotic-based therapeutics. A decline in the quantity and activity of *Lactobacillus* leads to an overgrowth of anaerobic bacteria. Deleterious metabolites such as nitrous acid can be produced by these organisms, and the risk of HPV infection also increases. Persistent infection of oncogenic HPV is a cause of cervical cancer. The abundance of *Lactobacillus* in HPV infection is lower, and HPV oncogenes may be involved in regulation of the viability of cervical cancer cells inhibited by *Lactobacillus*. Thus, the regulation and control of *Lactobacillus* may block the progression of cervical cancer. Many clinical studies found that a decline in the quantity and activity of *Lactobacillus* was involved in the initiation and progression of cervical cancer, which provides a novel insight into the use of probiotics to prevent cervical cancer. The continuation of research on the anti-cancer functions of specific probiotic strains and their mechanisms of action, especially during treatment, is therefore very necessary and beneficial. In order to understand the functional impact of probiotics of specific bacterial species in the treatment of cancer, more study is required. But to explore the other pathways of tumor inhibition, further research is needed.

Authorship

KK and PB collected the data wrote the article JSN and VB designed the content and supervised all stages. All authors critically reviewed the manuscript and approved the final manuscript.

Funding

No funding was received for this work.

Declaration of competing interest

None declared.

Acknowledgements

We are very thankful to Dr. Buddolla's Institute of Life Sciences for supporting us in writing this review article.

References

[1] Simms KT, Steinberg J, Caruana M, Smith MA, Lew JB, Soerjomataram I, et al. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020–99: a modelling study. Lancet Oncol 2019;20(3):394–407.
[2] Rajoka MSR, Zhao H, Lu Y, Lian Z, Li N, Hussain N, et al. Anticancer potential against cervix cancer (HeLa) cell line of probiotic *Lactobacillus casei* and *Lactobacillus paracasei* strains isolated from human breast milk. Food Funct 2018;9(5):2705–15.
[3] Johnson CA, James D, Marzan A, Armaos M. Cervical cancer: an overview of pathophysiology and management. In: Seminars in oncology nursing. vol. 35. WB Saunders; 2019, April. p. 166–74. 2.
[4] Schiffman M, Doorbar J, Wentzensen N, De Sanjosé S, Fakhry C, Monk BJ, et al. Carcinogenic human papillomavirus infection. Nat Rev Dis Prim 2016;2(1):1–20.
[5] Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. Lancet 2019;393(10167):169–82.
[6] Liu J, Luo M, Zhang Y, Cao G, Wang S. Association of high-risk human papillomavirus infection duration and cervical lesions with vaginal microbiota composition. Ann Transl Med 2020;8(18):1161.
[7] Yang J, Wang W, Wang Z, Wang Z, Wang Y, Wang J, et al. Prevalence, genotype distribution and risk factors of cervical HPV infection in Yangqu, China: a population-based survey of 10086 women. Hum Vaccines Immunother 2020;16(7):1645–52.
[8] Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev 2003;16(1):1–17.
[9] Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev 2012;25(2):215–22.
[10] Brenna SMF, Syrjänen KJ. Regulation of cell cycles is of key importance in human papillomavirus (HPV)-associated cervical carcinogenesis. Sao Paulo Med J 2003;121(3):128–32.
[11] Chen J. Signaling pathways in HPV-associated cancers and therapeutic implications. Rev Med Virol 2015;25:24–53.
[12] Tsuda N, Watari H, Ushijima K. Chemotherapy and molecular targeting therapy for recurrent cervical cancer. Chin J Cancer Res 2016;28(2):241.
[13] Jahanshahi M, Dana PM, Badehnoosh B, Asemi Z, Hallajzadeh J, Mansournia MA, et al. Anti-tumor activities of probiotics in cervical cancer. J Ovarian Res 2020;13(1):1–11.
[14] Best SR, Niparko KJ, Pai SI. Biology of human papillomavirus infection and immune therapy for HPV-related head and neck cancers. Otolaryngol Clin 2012;45(4):807–22.
[15] Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J 2017;474(11):1823–36.
[16] Mercenier A, Pavan S, Pot B. Probiotics as biotherapeutic agents: present knowledge and future prospects. Curr Pharmaceut Des 2003;9(2):175–91.
[17] Amabebe E, Anumba DO. The vaginal microenvironment: the physiologic role of lactobacilli. Front Med 2018;5:181.
[18] Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell 2014;157(1):121–41.
[19] Kaur H, Merchant M, Haque MM, Mande SS. Crosstalk between female gonadal hormones and vaginal microbiota across various phases of women's gynecological lifecycle. Front Microbiol 2020;11:551.
[20] Amara AA, Shibl A. Role of Probiotics in health improvement, infection control and disease treatment and management. Saudi Pharmaceut J 2015;23(2):107–14.
[21] Dos Reis SA, da Conceição LL, Siqueira NP, Rosa DD, da Silva LL, Maria do Carmo GP. Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. Nutr Res (NY) 2017;37:1–19.
[22] Wang KD, Xu DJ, Wang BY, Yan DH, Lv Z, Su JR. Inhibitory effect of vaginal *Lactobacillus* supernatants on cervical cancer cells. Probiotics and antimicrobial proteins 2018;10(2):236–42.
[23] Pourmollaei S, Barzegari A, Farshbaf-Khalili A, Nouri M, Fattahi A, Shahnazi M, et al. Anticancer effect of bacteria on cervical cancer: molecular aspects and therapeutic implications. Life Sci 2020;246:117413.
[24] Amara A. Toward healthy genes. Münster: Schüling Verlage; 2012.
[25] Hosono A, Nakazawa Y, editors. Functions of fermented milk: challenges for the health Sciences. Elsevier Applied Science; 1992.
[26] Metchnikoff E, Mitchell C. Nature of man or studies in optimistic philosophy. Yew York and London: GP Putnam's Son; 1910.
[27] Metchnikoff E, Mitchell PC. Prolongation of life: optimistic studies (classics in longevity and aging series). Springer Publishing Company; 2004.
[28] Parker RB. Probiotics, the other half of the antibiotic story. Anim Nutr Health 1974;29:4–8.
[29] Uccello M, Malaguarnera G, Basile F, D'agata V, Malaguarnera M, Bertino G, et al. Potential role of probiotics on colorectal cancer prevention. BMC Surg 2012;12(1):1–8.
[30] Liong MT, editor. Probiotics: biology, genetics and health aspects, vol. 21. Springer Science & Business Media; 2011.
[31] Patel S, Goyal A. Evolving roles of probiotics in cancer prophylaxis and therapy. Probiotics and antimicrobial proteins 2013;5(1):59–67.
[32] Abdolalipour E, Mahooti M, Salehzadeh A, Torabi A, Mohebbi SR, Gorji A, et al. Evaluation of the antitumor immune responses of probiotic *Bifidobacterium bifidum* in human papillomavirus-induced tumor model. Microb Pathog 2020;145:104207.
[33] Nazir Y, Hussain SA, Abdul Hamid A, Song Y. Probiotics and their potential preventive and therapeutic role for cancer, high serum cholesterol, and allergic and HIV diseases. BioMed Res Int 2018:3428437. https://doi.org/10.1155/2018/3428437. 2018.
[34] Dasari S, Kathera C, Janardhan A, Kumar AP, Viswanath B. Surfacing role of probiotics in cancer prophylaxis and therapy: a systematic review. Clin Nutr 2017;36(6):1465–72.
[35] Rossi M, Keshavarzian A, Bishehsari F. Nutraceuticals in colorectal cancer: a mechanistic approach. Eur J Pharmacol 2018;833:396–402.
[36] Sharma A, Viswanath B, Park YS. Role of probiotics in the management of lung cancer and related diseases: an update. J Funct Foods 2018;40:625–33.
[37] Reid R, Stanhope CR, Herschman BR, Booth E, Phibbs GD, Smith JP. Genital warts and cervical cancer. I. Evidence of an association between subclinical papillomavirus infection and cervical malignancy. Cancer 1982;50(2):377–87.
[38] Insinga RP, Dasbach EJ, Elbasha EH. Epidemiologic natural history and clinical management of Human Papillomavirus (HPV) Disease: a critical and systematic review of the literature in the development of an HPV dynamic transmission model. BMC Infect Dis 2009;9(1):1–26.
[39] Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol 2010;117(2):S5–10.
[40] Rachel Skinner S, Wheeler CM, Romanowski B, Castellsagué X, Lazcano-Ponce E, Rowena Del Rosario-Raymundo M, VIVIANE Study Group. Progression of HPV infection to detectable cervical lesions or clearance in adult women: analysis of the control arm of the VIVIANE study. Int J Cancer 2016;138(10):2428–38.
[41] Brianti P, De Flammineis E, Mercuri SR. Review of HPV-related diseases and cancers. New Microbiol 2017;40(2):80–5.
[42] Chan CK, Aimagambetova G, Ukybassova T, Kongrtay K, Azizan A. Human papillomavirus infection and cervical cancer: epidemiology, screening, and vaccination—review of current perspectives. J oncol 2019;2019:3257939. https://doi.org/10.1155/2019/3257939.
[43] Lehoux M, D'Abramo CM, Archambault J. Molecular mechanisms of human papillomavirus-induced carcinogenesis. Public health genomics 2009;12(5–6):268–80.
[44] Jing Y, Wang T, Chen Z, Ding X, Xu J, Mu X, Chen H. Phylogeny and polymorphism in the long control regions E6, E7, and L1 of HPV Type 56 in women from southwest China. Mol Med Rep 2018;17(5):7131–41.
[45] Lee SH, eun Kwon J, Cho ML. Immunological pathogenesis of inflammatory bowel disease. Intestinal research 2018;16(1):26.
[46] Drago L. Probiotics and colon cancer. Microorganisms 2019;7(3):66.
[47] Sundararaman A, Ray M, Ravindra PV, Halami PM. Role of probiotics to combat viral infections with emphasis on COVID-19. Appl Microbiol Biotechnol 2020;104:8089–104.
[48] Rajoka MSR, Shi J, Mehwish HM, Zhu J, Li Q, Shao D, et al. Interaction between diet composition and gut microbiota and its impact on gastrointestinal tract health. Food Science and Human Wellness 2017a;6(3):121–30.
[49] Audirac-Chalifour A, Torres-Poveda K, Bahena-Román M, Téllez-Sosa J, Martínez-Barnetche J, Cortina-Ceballos B, et al. Cervical microbiome and cytokine profile at various stages of cervical cancer: a pilot study. PLoS One 2016;11(4):e0153274.
[50] Rajoka MSR, Shi J, Zhu J, Shao D, Huang Q, Yang H, et al. Capacity of lactic acid bacteria in immunity enhancement and cancer prevention. Appl Microbiol Biotechnol 2017b;101(1):35–45.
[51] Verhoeven V, Renard N, Makar A, Van Royen P, Bogers JP, Lardon F, et al. Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study. Eur J Cancer Prev 2013;22(1):46–51.

[52] Linn YH, Thu KK, Win NHH. Effect of probiotics for the prevention of acute radiation-induced diarrhoea among cervical cancer patients: a randomized double-blind placebo-controlled study. Probiotics and antimicrobial proteins 2019;11(2):638–47.
[53] Curty G, de Carvalho PS, Soares MA. The role of the Cervicovaginal microbiome on the genesis and as a biomarker of premalignant cervical intraepithelial neoplasia and invasive cervical Cancer. Int J Mol Sci 2020;21(1):222.
[54] Ou YC, Fu HC, Tseng CW, Wu CH, Tsai CC, Lin H. The influence of probiotics on genital high-risk human papilloma virus clearance and quality of cervical smear: a randomized placebo-controlled trial. BMC Wom Health 2019;19(1):103.
[55] Kyrgiou M, Mitra A, Moscicki AB. Does the vaginal microbiota play a role in the development of cervical cancer? Transl Res 2017;179:168–82.
[56] Bedell SL, Goldstein LS, Goldstein AR, Goldstein AT. Cervical cancer screening: past, present, and future. Sexual medicine reviews 2020;8(1):28–37.
[57] Nami Y, Abdullah N, Haghshenas B, Radiah D, Rosli R, Khosroushahi AY. Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium *Lactobacillus plantarum* 5BL. Microbiol Immunol 2014;58(9):492–502.
[58] Sungur T, Aslim B, Karaaslan C, Aktas B. Impact of Exopolysaccharides (EPSs) of *Lactobacillus gasseri* strains isolated from human vagina on cervical tumor cells (HeLa). Anaerobe 2017;47:137–44.
[59] Nouri Z, Karami F, Neyazi N, Modarressi MH, Karimi R, Khorramizadeh MR, et al. Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. Cell Journal (Yakhteh) 2016;18(2):127.
[60] Górska A, Przystupski D, Niemczura MJ, Kulbacka J. Probiotic bacteria: a promising tool in cancer prevention and therapy. Curr Microbiol 2019;76(8):939–49.
[61] Motevaseli E, Dianatpour A, Ghafouri-Fard S. The role of probiotics in cancer treatment: emphasis on their in vivo and in vitro anti-metastatic effects. International journal of molecular and cellular medicine 2017;6(2):66.
[62] Li X, Wang H, Du X, Yu W, Jiang J, Geng Y, et al. *Lactobacilli* inhibits cervical cancer cell migration in vitro and reduces tumor burden in vivo through upregulation of E-cadherin. Oncol Rep 2017;38(3):1561–8.
[63] Cha MK, Lee DK, An HM, Lee SW, Shin SH, Kwon JH, et al. Antiviral activity of *Bifidobacterium* adolescentis SPM1005-A on human papillomavirus type 16. BMC Med 2012;10(1):1–6.
[64] Gui QF, Lu HF, Zhang CX, Xu ZR, Yang YH. Well-balanced commensal microbiota contributes to anti-cancer response in a lung cancer mouse model. Genet Mol Res 2015;14(2):5642–51.
[65] Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, Goldszmid RS. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013;342(6161):967–70.
[66] Zitvogel L, Daillère R, Roberti MP, Routy B, Kroemer G. Anticancer effects of the microbiome and its products. Nat Rev Microbiol 2017;15(8):465.
[70] Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic mechanisms of action. Ann Nutr Metabol 2012;61(2):160–74.
[71] Gauri A, Morya S. Probiotics as therapeutics. J Adv Res Biotech 2017;2(3):1–6.
[72] Stein A, Voigt W, Jordan K. Chemotherapy-induced diarrhea: pathophysiology, frequency and guideline-based management. Therapeutic advances in medical oncology 2010;2(1):51–63.
[73] Chitapanarux I, Chitapanarux T, Traisathit P, Kudumpee S, Tharavichitkul E, Lorvidhaya V. Randomized controlled trial of live *lactobacillus acidophilus* plus *bifidobacteriumbifidum* in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. Radiat Oncol 2010;5(1):1–6.
[74] Andreyev HJN, Davidson SE, Gillespie C, Allum WH, Swarbrick E. Practice guidance on the management of acute and chronic gastrointestinal problems arising as a result of treatment for cancer. Gut 2012;61(2):179–92.
[75] Maroun JA, Anthony LB, Blais N, Burkes R, Dowden SD, Dranitsaris G, et al. Prevention and management of chemotherapy-induced diarrhea in patients with colorectal cancer: a consensus statement by the Canadian Working Group on Chemotherapy-Induced Diarrhea. Curr Oncol 2007;14(1):13.
[76] Juntunen M, Kirjavainen PV, Ouwehand AC, Salminen SJ, Isolauri E. Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. Clin Diagn Lab Immunol 2001;8(2):293–6.
[77] Qiu G, Yu Y, Wang Y, Wang X. The significance of probiotics in preventing radiotherapy-induced diarrhea in patients with cervical cancer: a systematic review and meta-analysis. Int J Surg 2019;65:61–9.
[78] Mego M, Holec V, Drgona L, Hainova K, Ciernikova S, Zajac V. Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy. Compl Ther Med 2013;21(6):712–23.
[79] Mansouri-Tehrani HS, Khorasgani MR, Roayaei M. Effects of probiotics with or without honey on radiation-induced diarrhea. Int J Radiat Res 2016;14(3):205.
[80] Österlund P, Ruotsalainen T, Korpela R, Saxelin M, Ollus A, Valta P, et al. *Lactobacillus* supplementation for diarrhoea related to chemotherapy of colorectal cancer: a randomised study. Br J Cancer 2007;97(8):1028–34.
[81] Liu Z, Qin H, Yang Z, Xia Y, Liu W, Yang J, et al. Randomised clinical trial: the effects of perioperative probiotic treatment on barrier function and post-operative infectious complications in colorectal cancer surgery–a double-blind study. Aliment Pharmacol Ther 2011;33(1):50–63.
[82] Shida K, Nomoto K. Probiotics as efficient immunopotentiators: translational role in cancer prevention. Indian J Med Res 2013;138(5):808.
[83] Tiptiri-Kourpeti, A., Spyridopoulou, K., Santarmaki, V., Aindelis, G., Tompoulidou, E., Lamprianidou, E. E., et al (2016).
[84] Vasiljevic T, Shah NP. Probiotics—from Metchnikoff to bioactives. Int Dairy J 2008;18(7):714–28.
[85] Tarrah, A., da Silva Duarte, V., de Castilhos, J., Pakroo, S., Junior, W. J. F. L., Luchese, R. H., ... & Giacomini, A. (2019)..
[86] Wei D, Heus P, van de Wetering FT, van Tienhoven G, Verleye L, Scholten RJ. Probiotics for the prevention or treatment of chemotherapy-or radiotherapy-related diarrhoea in people with cancer. Cochrane Database Syst Rev 2018;(8).
[87] Chichlowski M, Hale LP. Bacterial-mucosal interactions in inflammatory bowel disease—an alliance gone bad. Am J Physiol Gastrointest Liver Physiol 2008;295(6):G1139–49.
[88] Lapointe TK, O'connor PM, Buret AG. The role of epithelial malfunction in the pathogenesis of enteropathogenic E. coli-induced diarrhea. Lab Invest 2009;89(9):964–70.
[89] Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448(7152):427–34.
[90] McGuckin MA, Eri R, Simms LA, Florin TH, Radford-Smith G. Intestinal barrier dysfunction in inflammatory bowel diseases. Inflamm Bowel Dis 2009;15(1):100–13.
[91] Ohland CL, MacNaughton WK. Probiotic bacteria and intestinal epithelial barrier function. Am J Physiol Gastrointest Liver Physiol 2010;298(6):G807–19.
[92] Beachey EH. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. JID (J Infect Dis) 1981;143(3):325–45.
[93] Schiffrin EJ, Brassart D, Servin AL, Rochat F, Donnet-Hughes A. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. Am J Clin Nutr 1997;66(2):515S–20S.
[94] Perdigon G, Galdeano CM, Valdez JC, Medici M. Interaction of lactic acid bacteria with the gut immune system. Eur J Clin Nutr 2002;56(4):S21–6.
[95] Hirano J, Yoshida T, Sugiyama T, Koide N, Mori I, Yokochi T. The effect of *Lactobacillus rhamnosus* on enterohemorrhagic *Escherichia coli* infection of human intestinal cells in vitro. Microbiol Immunol 2003;47(6):405–9.
[96] Salminen S, Bouley C, Boutron MC, Cummings JH, Franck A, Gibson GR, Rowland I. Functional food science and gastrointestinal physiology and function. Br J Nutr 1998;80(S1):S147–71.
[97] Collado MC, Gueimonde M, Hernandez M, Sanz Y, Salminen S. Adhesion of selected *Bifidobacterium* strains to human intestinal mucus and the role of adhesion in enteropathogen exclusion. J Food Protect 2005;68(12):2672–8.
[98] Crociani J, Grill JP, Huppert M, Ballongue J. Adhesion of different *bifidobacteria* strains to human enterocyte-like Caco-2 cells and comparison with in vivo study. Lett Appl Microbiol 1995;21(3):146–8.
[99] Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. Curr Gastroenterol Rep 2010;12(5):319–30.
[100] Mishra V, Shah C, Mokashe N, Chavan R, Yadav H, Prajapati J. Probiotics as potential antioxidants: a systematic review. J Agric Food Chem 2015;63(14):3615–26.
[101] Wang S, Wang Q, Yang E, Yan L, Li T, Zhuang H. Antimicrobial compounds produced by vaginal *Lactobacillus crispatus* are able to strongly inhibit *Candida albicans* growth, hyphal formation and regulate virulence-related gene expressions. Front Microbiol 2017;8:564.
[102] Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol 2014;24(10):R453–62.
[103] Lin MY, Yen CL. Antioxidative ability of lactic acid bacteria. J Agric Food Chem 1999;47(4):1460–6.
[104] Shen Q, Shang N, Li P. In vitro and in vivo antioxidant activity of *Bifidobacterium* animalis 01 isolated from centenarians. Curr Microbiol 2011;62(4):1097–103.
[105] Persichetti E, De Michele A, Codini M, Traina G. Antioxidative capacity of Lactobacillus fermentum LF31 evaluated in vitro by oxygen radical absorbance capacity assay. Nutrition 2014;30(7–8):936–8.
[106] Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti–PD-L1 efficacy. Science 2015;350(6264):1084–9.
[107] Stecchini ML, Del Torre M, Munari M. Determination of peroxy radical-scavenging of lactic acid bacteria. Int J Food Microbiol 2001;64(1–2):183–8.
[108] Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H, Kairane C, et al. Two antioxidative *lactobacilli* strains as promising probiotics. Int J Food Microbiol 2002;72(3):215–24.
[109] Jounai K, Ikado K, Sugimura T, Ano Y, Braun J, Fujiwara D. Spherical lactic acid bacteria activate plasmacytoid dendritic cells immunomodulatory function via TLR9-dependent crosstalk with myeloid dendritic cells. PLoS One 2012;7(4):e32588.
[110] Nolfo F, Rametta S, Marventano S, Grosso G, Mistretta A, Drago F, Biondi A. Pharmacological and dietary prevention for colorectal cancer. BMC Surg 2013;13(2):1–11.
[111] Vanderpool C, Yan F, Polk BD. Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. Inflamm Bowel Dis 2008;14(11):1585–96.

[112] Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, Im SH. Generation of regulatory dendritic cells and CD4+ Foxp3+ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci Unit States Am 2010;107(5):2159–64.
[113] Hardy H, Harris J, Lyon E, Beal J, Foey AD. Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients 2013;5(6):1869–912.
[114] Jia W, Xie G, Jia W. Bile acid–microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nat Rev Gastroenterol Hepatol 2018;15(2):111.
[115] Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutat Res Rev Mutat Res 2005;589(1):47–65.
[116] Biasco G, Paganelli GM, Brandi G, Brillanti S, Lami F. Effect of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on rectal cell kinetics and fecal pH. Ital J Gastroenterol 1991;23(3).
[117] Lidbeck A, Allinger UG, Orrhage KM, Ottova L, Brismar B, Gustafsson JÅ, et al. Impact of *Lactobacillus acidophilus* supplements on the faecal microflora and soluble faecal bile acids in colon cancer patients. Microb Ecol Health Dis 1991;4(2):81–8.
[118] Okawa T, Niibe H, Arai T, Sekiba K, Noda K, Takeuchi S, Ogawa N. Effect of LC9018 combined with radiation therapy on carcinoma of the uterine cervix. A phase III, multicenter, randomized, controlled study. Cancer 1993;72(6):1949–54.
[119] Gueimonde M, Ouwehand AC, Salminen S. Safety of probiotics. Scand J Nutr 2004;48(1):42–8.
[120] Saarela M, Matto J, Mattila-Sandholm T. Safety aspects of *Lactobacillus* and *Bifidobacterium* species originating from human oro-gastrointestinal tract or from probiotic products. Microb Ecol Health Dis 2002;14(4):234–41.
[121] Mogensen G, Salminen S, O'brien J, Ouwenhand A, Holzapfel W, Shortt C, Zink R. Food microorganisms-health benefits, safety evaluation and strains with documented history of use in foods. 2002.

Chapter 7

Middle East respiratory syndrome: outbreak response priorities, treatment strategies, and clinical management approaches

Kandati Kusuma[1,2], Pandeeti Emmanuel Vijay Paul[3] and Buddolla Viswanath[2]

[1]Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, India, [2]Dr. Buddolla's Institute of Life Sciences, A Unit of Dr. Buddolla's Educational Society, Tirupati, India, [3]Albus Eco Projects, LLP, BioNEST, University of Hyderabad, Hyderabad, India

7.1 Introduction

Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by a novel coronavirus (Middle East respiratory syndrome coronavirus, or MERS-CoV), which was initially discovered in Saudi Arabia in 2012 and it is one of six known human coronaviruses that cause respiratory disease in humans and, with a mortality rate >35% [1]. It is the first highly pathogenic human coronavirus to emerge since the global scare caused by the severe acute respiratory syndrome coronavirus in 2003 [2]. The first human MERS-CoV infection was found in Saudi Arabia, in June 2012 in a 60-year old man who developed renal failure [3]. Analysis revealed the disease is due to a novel virus which was named Middle East Respiratory Coronavirus [4]. The World Health Organization [5] has confirmed 2279 cases of human infections with MERS-CoV in 27 countries since 2012; 806 (35%) infected patients have died as of February 13, 2019 [6]. However, Saudi Arabia still has the highest reported MERS-CoV mortality rate where approximately 80% of the cases have been reported to occur there [7,8]. MERS-CoV remains a high-threat pathogen identified by WHO as a priority pathogen because it causes severe disease that has a high mortality rate, epidemic potential, and no medical countermeasures [9]. MERS-CoV belongs to the family *Coronaviridae*, order *Nidovirales*. It is one of the recently reported zoonotic viruses [10]. The family *Coronaviridae* is classified into four genera (α, β, γ, and δ). Each genus is divided into lineage subgroups. MERS-CoV belongs to lineage-C of the β coronaviruses [11]. Viral spread has been observed among healthcare workers and among individuals visiting MERS-CoV-positive patients. The control of some of these outbreaks has been achieved by the local center for disease control and prevention (CDC). Respiratory tract infections are the leading cause of mortality in resource-limited settings, accounting for more than 4 million deaths each year globally [12]. A hypothetical sequence of how humans and DCs (direct contact) lead to the spread of MERS cases is summarized in Fig. 7.1.

MERS-CoV infection may be implicated in transmission. As an emerging Betacoronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV) causes illness characterized predominantly by mild-to-severe respiratory complaints, with most patients requiring admission to hospital because of pneumonitis or acute respiratory distress syndrome. Old age and the presence of comorbidities or immunosuppression seem to increase the risk of infection and are associated with severe forms of the disease [14].

Humans are thought to acquire MERS-CoV through contact with camels or camel products [15]. Despite the increase in the number of cases, the actual incidence of MERS-CoV among hospitalized patients with community-acquired pneumonia is low [16]. There are reports of the role of asymptomatic individuals in the transmission of MERS-CoV; however, the exact role is not known [16]. These observations indicate the need for understanding the human immune response to the virus to guide immunotherapy of severely ill patients and vaccine development and to

Pandemic Outbreaks in the 21st Century. DOI: https://doi.org/10.1016/B978-0-323-85662-1.00016-1

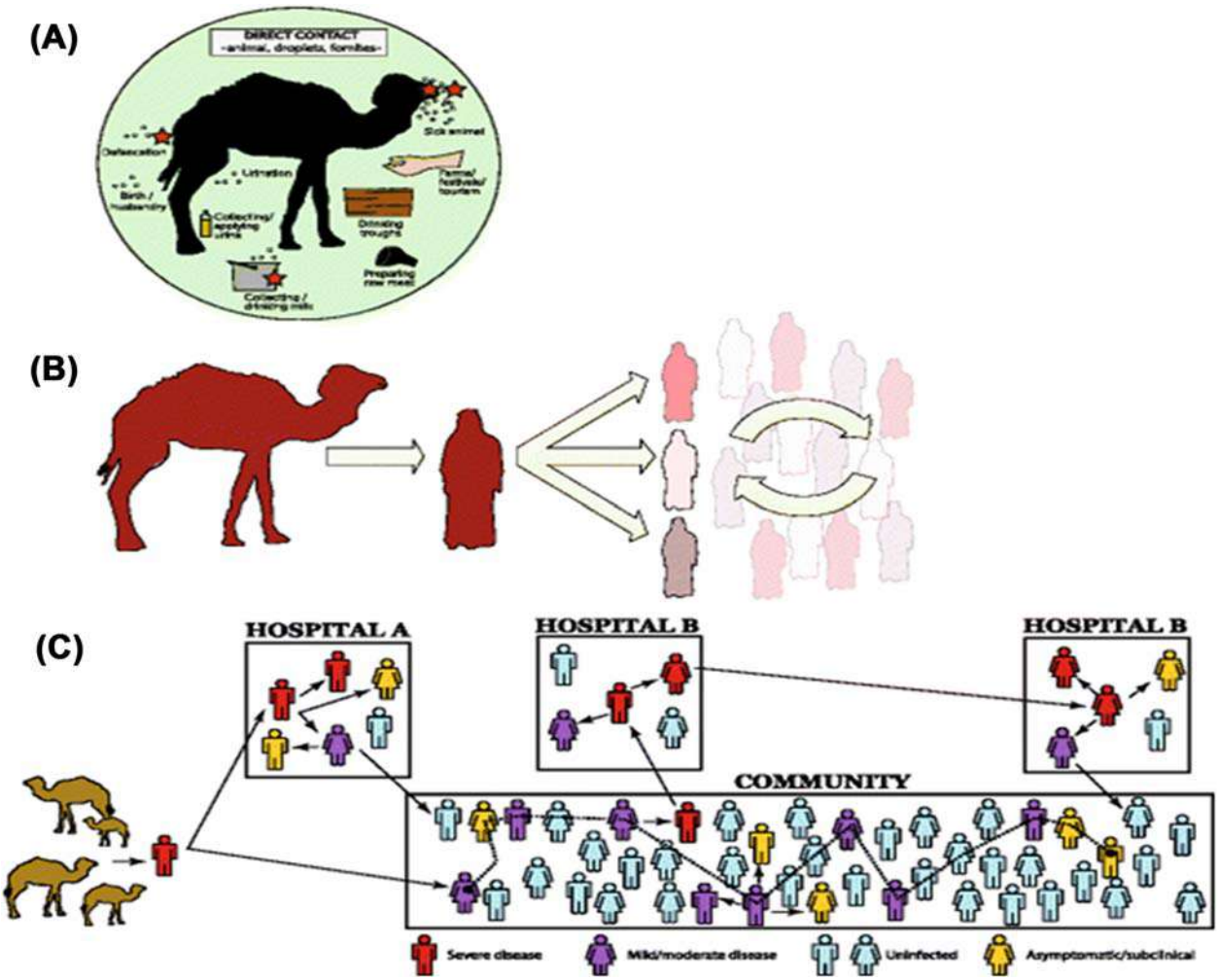

FIGURE 7.1 (A) Risks for acquiring MERS-CoV from a DC. This illustration highlights risks that may originate from a droplet transmission component (be they larger, heavier wet droplets or the drier, airborne gel-like droplet nuclei) or a direct contact component (within the *green circle*). No routes of MERS-CoV acquisition to or between humans have been proven to date [13]. (B) Camel-to-human infections appear to be infrequent, while human-to-human spread of infection is regularly facilitated by poor IPC in healthcare settings where transmission is amplified, accounting for the bulk of cases. (C) A hypothetical sequence of how humans and direct contact (DCs) lead to the spread of MERS cases. *MERS-CoV*, Middle East respiratory syndrome coronavirus; *DC*, dendritic cells.

develop additional tools for determining the prevalence of the infection [17]. MERS-CoV causes acute, highly lethal pneumonia and renal dysfunction with various clinical symptoms, including but not restricted to fever, cough, sore throat, myalgia, chest pain, diarrhea, vomiting, and abdominal pain [18]. This chapter focuses on the current information of MERS-CoV, epidemiology, and spreading of MERS-CoV virus with reference to the virus structure and life cycle, molecular mechanisms of pathogenesis, and immune responses to MERS infection. We also look at the initial and postinfection manifestations of MERS and its future prospects as well.

7.2 Epidemiology

Molecular clock dating of epidemiologically unlinked human MERS-CoV isolates estimated their divergence from a common ancestor in mid-2011, with a cluster of isolates from the eastern parts of the Arabian Peninsula diverging in late 2012. These findings suggest that the reported MERS-CoV diversity in human beings is the result of several independent, geographically structured, zoonotic events from an unknown reservoir in the Middle East [19,20]. A primary MERS-CoV infection is defined by WHO as laboratory-confirmed MERS-CoV infection that has no direct epidemiological link to a human MERS-CoV infection and was acquired outside of a healthcare facility presumably from direct or indirect contact

with the reservoir host, dromedary camels. A secondary MERS-CoV infection is defined by WHO as a laboratory-confirmed MERS-CoV infection with a direct epidemiological link to an individual with confirmed or probable MERS-CoV infection [9]. MERS-CoV cases continued to be reported from the community and hospitals across the Arabian Peninsula. As of July 31, 2019, 2458 laboratory-confirmed MERS cases were reported to WHO of which there were 848 deaths (34% mortality) [15]. Approximately 80% of human cases have been reported in Saudi Arabia. Twenty-seven countries have reported cases of MERS [21]. Countries in or near the Arabian Peninsula that report MERS cases are Bahrain, Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, UAE, and Yemen. Cases identified outside the Middle East are usually in travelers who were infected in the Middle East and then traveled to areas outside the Middle East. Countries outside the Arabian Peninsula that have reported travel-associated MERS cases are Algeria, Austria, China, Egypt, France, Germany, Greece, Italy, Malaysia, Netherlands, Philippines, Republic of Korea, Thailand, Tunisia, Turkey, United Kingdom, and the United States [15]. Serologic surveys subsequently conducted in several countries in the Arabian Peninsula and Africa identified high rates of MERS-CoV−specific antibodies in dromedary camels [22,23]. Furthermore, MERS-CoV infection in dromedary camels was definitively proven by the detection of virus and virus sequences in respiratory specimens, feces, and milk collected from camels in Qatar [14,24], Oman [25], Saudi Arabia [22,26−28], and Egypt [29].

7.3 Ecology and spreading of MERS-CoV virus

MERS-CoV is a virus transferred to humans from infected dromedary camels. It is a zoonotic virus, meaning it is transmitted between animals and people, and it is contractible through direct or indirect contact with infected animals. MERS-CoV has been identified in dromedaries in several countries in the Middle East, Africa, and South Asia. In total, 27 countries have reported cases since 2012, leading to 858 known deaths due to the infection and related complications [5]. For MERS-CoV, however, increasing evidence indicates that both camels and humans may play intermediate roles, as both disease victims and reservoirs for further transmission [30]. Transmission appears to be entirely from humans to humans [3]. Contact of various types with camels, such as consumption of raw milk, butchering and cleaning meat, and visiting live animals, has been identified as a significant risk factor, such that camels are seen as a significant reservoir host for MERS-CoV in its transmission to humans [31].

Cases of MERS-CoV can be found in countries like America, UK, France, Tunisia, Italy, Malaysia, Philippines, Greece, Egypt, the Netherlands, Algeria, Austria, Turkey, and so on whose citizens have traveled to the Middle East [3]. On May 20, 2014, a man at the age of 68 was the first to be diagnosed with MERS-CoV in Korea. He traveled to Bahrain, Saudi Arabia, and Qatar for 16 days. On May 4, 2015, this patient entered Korea, and febrile sense and respiratory symptoms appeared on May 11. He visited Clinic A on the day and was admitted to Hospital B from May 15 to 17. Since the symptoms worsened, he visited Clinic C on May 18, and finally he was transferred to a university hospital in Seoul on May 18. On May 20, it was confirmed that he was suffering from MERS-CoV. After finding out about the disease, his family members and medical staff who had been exposed to the virus were isolated. By June 9, 2015, two medical staffs in the Clinics A and C, one medical staff in the Hospital B, one patient and his wife who was together with the index case in the same room and 35 of admitted patients in the same ward and their family members visiting the same ward with the index case in the Hospital B were confirmed to have been infected with MERS-CoV. After then, several tertiary cases were identified in the Hospital B or other hospitals that secondary patients were transferred from the Hospital B. A total of 108 people were infected, and nine (8.3%) of them died by June 10, 2015. The ecology and transmission of MERS-CoV are pictured in Fig. 7.2.

MERS-CoV might have originally spread from bats to camels and others, as yet unidentified, intermediate hosts. The virus has circulated in camel populations in Africa and the Arabian Peninsula for at least 20 years. In 2012 MERS-CoV spread to human populations, with camels the most likely source. Several possible routes of spread from camels to humans exist. MERS-CoV is believed to be transmitted among human beings by droplet, contact, and perhaps airborne spread. MERS manifests in people in various ways, ranging from asymptomatic to fulminant infections. Patients with underlying diseases such as diabetes or kidney or liver disease or who are immunocompromised develop more severe diseases and have a higher mortality rate after infection [32].

7.4 Virus structure and life cycle

The Middle East respiratory syndrome (MERS) is a highly lethal respiratory disease caused by a novel single-stranded, positive-sense RNA *Betacoronavirus* (MERS-CoV) [32]. MERS-CoV belongs to the genus *Betacoronavirus* of the Coronaviridae family [33]. It is an enveloped, single-stranded, positive-sense RNA virus with a helical capsid structure.

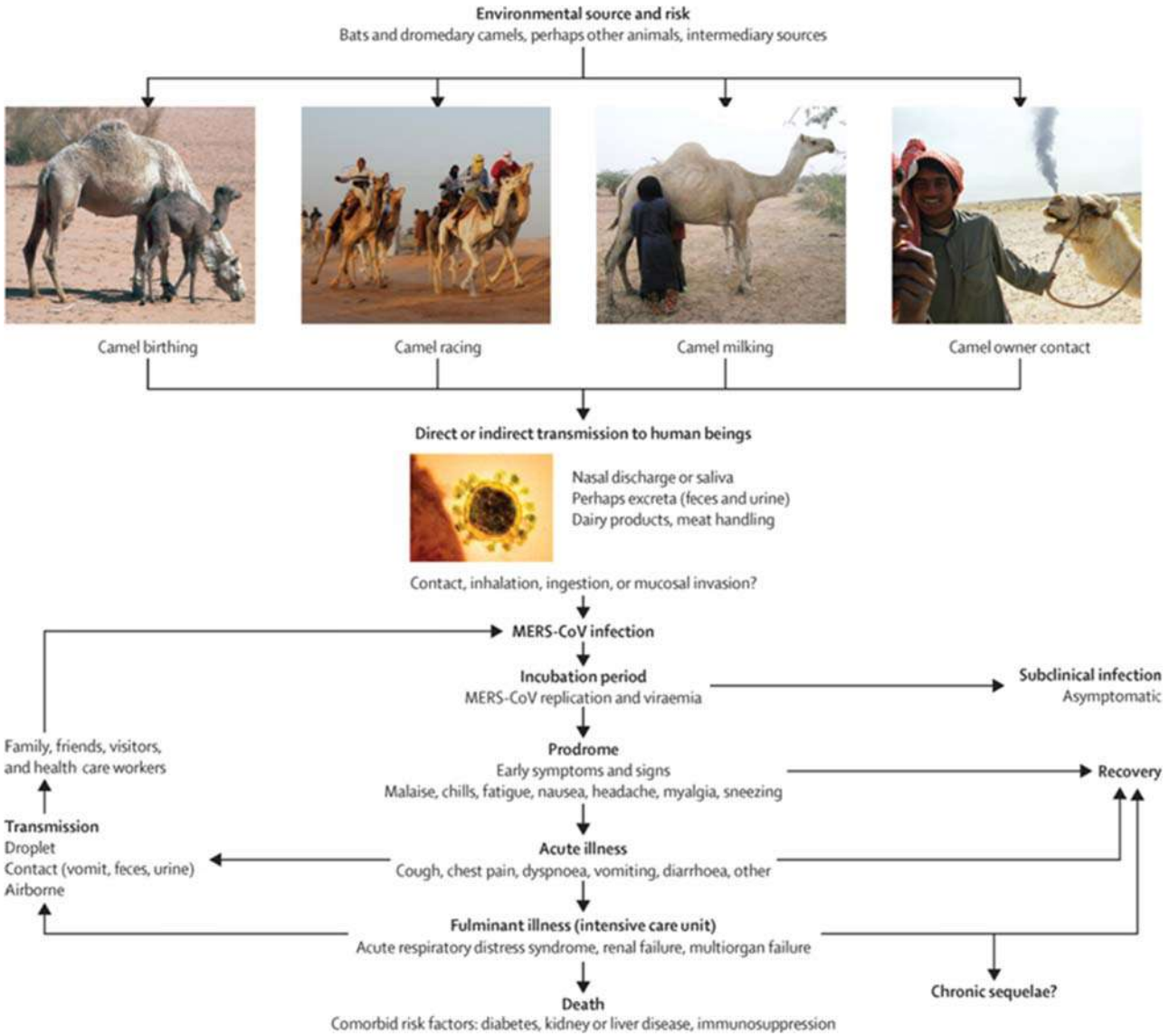

FIGURE 7.2 Ecology and transmission of MERS-CoV. *MERS-CoV*, Middle East respiratory syndrome coronavirus. *Reprinted with permission from Elsevier, Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. Lancet 2015;386(9997):995–1007.*

The genome of MERS-CoV is around 30 kb (30,119 nt) long one of the largest among positive-sense RNA viruses and encodes four structural proteins (spike, envelope, membrane, and nucleocapsid) and 16 nonstructural proteins depicted in Fig. 7.3 [34]. The immunogenic MERS-CoV proteins include spike (S), membrane (M), and envelope (E). Among them, the S protein mediates viral entry into the host cells [35]. The virion surface is covered with the spike glycoprotein, a 149 kDa glycoprotein that extends outward to create a crown-like appearance. The spike glycoprotein is critical for binding the host-cell receptor, dipeptidyl peptidase 4 (DPP-4), to initiate infection [36].

For MERS-CoV infection of humans, the primary receptor is a multifunctional cell surface protein, DPP4 (also known as CD26) [37] which is widely expressed on epithelial cells in the kidney, alveoli, small intestine, liver, and prostate, and on activated leukocytes (Widagdo et al., 2016). To enter host cells, MERS-CoV attaches to its receptor, DPP4. The S glycoprotein is a typical type I membrane glycoprotein consisting of a globular S1 domain at the N-terminal, followed by a membrane-proximal S2 domain and a transmembrane domain [39]. The S1 domain mediates viral attachment and contains the receptor binding domain (RBD), which determines the host range and cellular tropism for MERS-CoV [40–42].

Binding between RBD and the cell receptor (DPP4) triggers the conformational change of S glycoprotein to form a prehairpin intermediate of S2, in which the hydrophobic HR1 is exposed and the fusion peptide inserts into the target

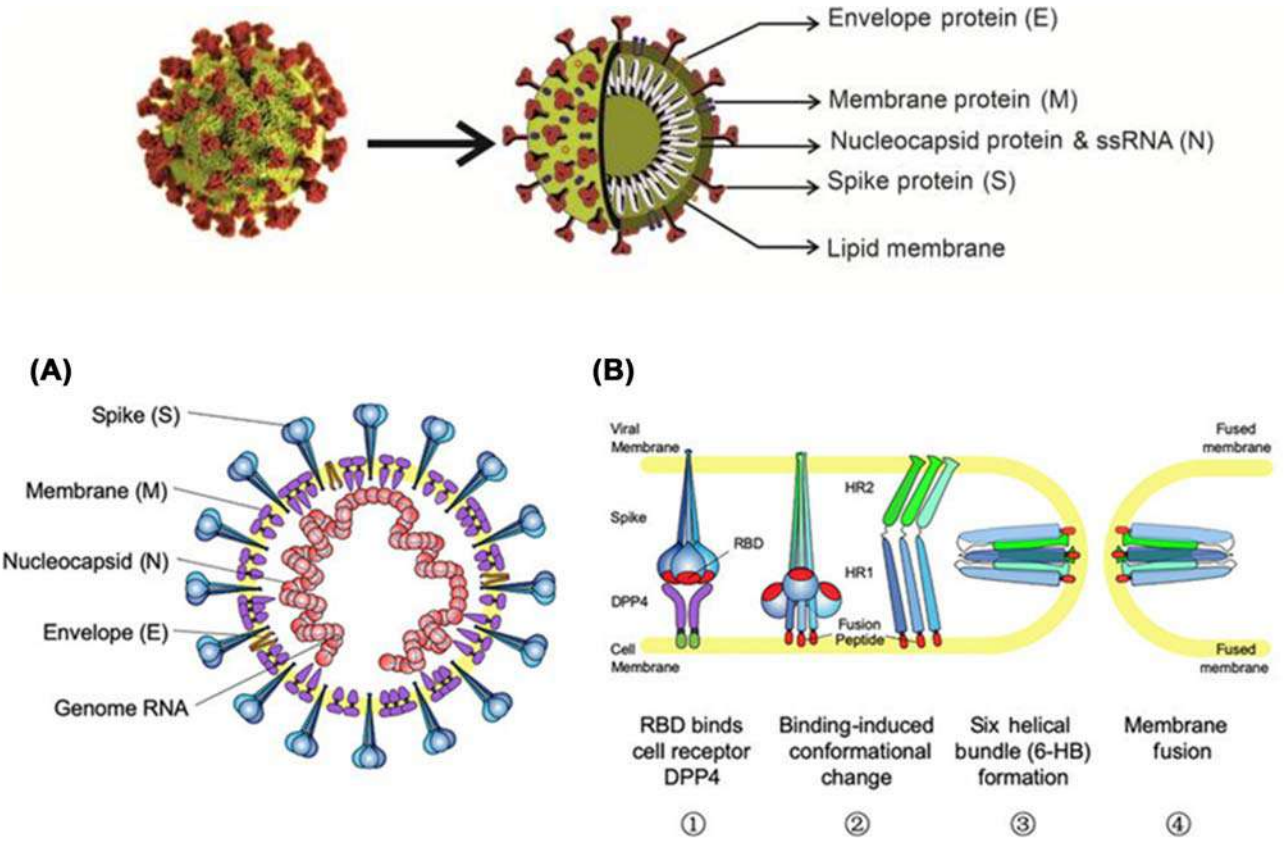

FIGURE 7.3 General structure and life cycle of MERS-CoV. (A) Cartoon model structure of MERS-CoV. (B) Membrane fusion mechanism for MERS-CoV spike glycoprotein. *MERS-CoV*, Middle East respiratory syndrome coronavirus.

cell membrane. This transient S2 intermediate then refolds with HR2 into a stabilized trimer of hairpins, also called six-helix bundle structure, bringing the target cell membrane into close proximity of the viral envelope and resulting in the completion of the fusion process [43]. Protease cleavage of the S protein is then required for virus−cell fusion and release of genomic RNA into the cytoplasm. Viral RNA transcription and replication occur on double-membrane vesicles and other membranous structures, which are derived from the endoplasmic reticulum. Transcription of the seven subgenomic mRNAs occurs via negative-strand subgenomic RNA intermediates. Subgenomic RNAs are 3′ coterminally nested and are joined to a common leader encoded at the 5′ end of the genome. Viral RNA is encapsulated in the N protein and transported to the endoplasmic reticulum−Golgi intermediate compartment, the site of assembly. Viral RNA encapsidated in the N protein then buds into vesicles lined with the S, M, and E proteins. Vesicles are then transported to the cell surface before release [32]. MERS-CoV binds to its cellular receptor DPP4 via the S protein, which is processed by host proteases to expose a fusion peptide. The viral genome is then released into the cytoplasm, where it is translated on host ribosomes into rep1A and rep1B proteins. The polyprotein is cleaved by two viral-encoded proteases, encoded by NSP3 and NSP5. Proteins involved in the genome and subgenome replication and transcription include nsp12 [the RNA-dependent RNA polymerase (RdRP)] and two associated proteins, NSP7 and NSP8 [44].

7.5 Molecular mechanisms of pathogenesis

Dromedary camels are infected with the virus and are believed to be the most likely source of animal-to-human transmission [45]. After intratracheal inoculation of MERS-CoV in nonhuman primates, the virus infects bronchial epithelial cells through DPP-4 before spreading to lung parenchymal cells, including type I and type II alveolar pneumocytes and endothelial cells. Viral entry is facilitated by another cell-surface protein, carcinoembryonic antigen−related cell-adhesion molecule 5, which is also expressed in lung tissue. Inflammatory signaling molecules that are released by infected cells, alveolar macrophages, and neutrophils recruited to infected tissue have been detected in infected patients

and animal models. A host antiviral type I and type III interferon response occurs, with systemic release of pro-inflammatory cytokines and chemokines [46–48].

The virus may spread into the circulation, possibly from lung parenchyma or through infected endothelial cells. In humans, a high viral copy number has been detected in the lower respiratory tract, including tracheal aspirates and bronchoalveolar lavage specimens, as well as in peripheral blood. In advanced disease, diffuse alveolar damage is seen, with extensive hemorrhagic edema and hyaline membrane deposition. CXCL10 denotes C-X-C motif chemokine 10, IL—interleukin, IL-1RA—IL-1 receptor antagonist, IFN—interferon, and MCP—monocyte chemotactic protein. In one study conducted in Saudi Arabia, the rate of MERS-CoV seropositivity was 15 times as high in shepherds and 23 times as high in slaughterhouse workers as in the general population [49]. There is high sequence homology between the viruses from sporadic cases in humans and the implicated dromedaries. Although the routes of transmission remain unclear, they appear to include contact with infectious nasal or other bodily secretions and possibly the consumption of raw dromedary products (e.g., unpasteurized milk). Although dromedary-to-human transmission of MERS-CoV is now well recognized, direct exposure to dromedaries has been documented in only 40% of primary cases [50].

Transmission of MERS-CoV between dromedary camels and humans has been documented in several countries. Human-to-human transmission in health care settings accounts for the majority of reported cases to date, although human-to-human transmission in household settings has also been identified [36].

7.6 Immune responses to MERS infection

The virus replicates in macrophages and dendritic cells (DC), it induces the production of pro-inflammatory cytokines. Infection of human T cells with MERS-CoV induces both intrinsic and extrinsic apoptotic pathways, causing the suppression of immune responses [51]. Neutrophils are considered the first line of the innate immune response. Studies on MERS-CoV infection revealed that neutrophil chemoattractant chemokine IL-8 is highly expressed in the lower respiratory tract of the patient [52]. IL-8 plays an essential role in recruitment, activation, and accumulation of neutrophil in the site of infection and subsequently induce the formation of neutrophil extracellular traps (NETs). NETs directly cause inflammation and increase the secretion of IL-8, resulting in the further recruitment of neutrophils to the site of infection [53].

The launch response of the immune system to the invading of a microorganism such as a virus is directly related to the host sensing of the target organism and its linked constituents like uncapped viral RNA or the cellular stress response and consequent biological changes or damages due to infection [54]. This response could be primarily conducted by germline-encoded pattern recognition receptors such as Toll-like receptors or Retinoic acid-inducible gene I-like from the components receptors (RLRs) that enable to detect pathogen-associated molecular patterns originated of a virus or its replication intermediates, promoting the initial antiviral signaling cascades in response to the infection [55]. MERS-CoV could infect airway epithelial cells, inducing the responses of pro-inflammatory cytokines like IL-1β, IL-6, IL-8, and IFNs significantly but delayed. Though MERS-CoV is able to be replicated in either naïve or activated DCs and monocyte-macrophages, activated T cells can only support the MERS-CoV replication [56]. The higher amount of these factors in the serum of MERS-infected patients is correlated with increased numbers of monocyte and neutrophil in the peripheral blood cells and lungs, showing the possible function of these cells in the pathology of the lungs [57]. Importantly, among all the functional/nonfunctional structural proteins of MERS-CoV, the S protein is the principal antigenic component that induces antibodies to block virus-binding, stimulate host immune responses, fuse or neutralize antibodies, and/or protect the immune system against virus infection. Therefore the S protein has been selected as a significant target for the development of vaccines [58].

7.7 MERS—initial and postinfection manifestations

The clinical manifestations of MERS are nonspecific, which include runny nose, sore throat, low-grade fever, and myalgia, before the viremia becomes detectable [36,59]. In case of severe disease, progression to acute respiratory distress may occur. Extrapulmonary manifestations including gastrointestinal symptoms and acute kidney failure have been reported in case of severe illness, as well as neurological manifestations [36]. The initial and postinfection manifestations associated with MERS-CoV-infection were observed to be related to neurological manifestations presenting neurological symptoms. Different neurological symptoms were reported from the studies on the patients infected with MERS-CoV. Patients with ataxia, vomiting, confusion were known to have acute disseminated encephalomyelitis or less probably encephalitis, whereas patients with unresponsiveness, hypotensive with left-sided facial paralysis showed acute bilaterally nonocclusive stroke probably due to MERS-CoV-vasculopathy, low Glasgow Coma Scale, and fever

were also found to be associated with encephalopathy [45]. Patients with right frontal lobe intracerebral hemorrhage showed symptoms of severe headache, nausea and vomiting, decreased level of consciousness and Glasgow Coma Scale; Weakness in both legs and inability to walk with numbness and tingling in stocking distribution were observed in critical illness polyneuropathy patients [60]. The above-mentioned neurological symptoms were more prominent in patients with comorbidities like hypertension, dyslipidemia, diabetes, peripheral vascular disease, chronic kidney disease, and ischemic heart disease [61].

7.8 Outbreak response priorities

Patients with MERS should be placed in negative pressure rooms or in rooms in which room exhaust is filtered through high-efficiency particulate air filters. Airborne precautions with at least six air changes per hour should be applied in treatment rooms when performing aerosol-generating procedures [62,63]. These recommendations are evidence-based and have proven to be effective in hospitals in affected countries. Camels infected with MERS-CoV can develop rhinitis or show no signs of infection and might shed the virus through nasal and eye discharge and feces. The virus can also be found in raw milk from infected camels. MERS-CoV is stable in camel breast milk for extended periods of time [64]. Thus pasteurization or cooking is recommended to destroy the virus. Camel farm workers, slaughterhouse workers, market workers, veterinarians, and those handling camels at racing facilities should practice good personal hygiene, including frequent hand washing after touching animals, avoiding touching eyes, nose, or mouth with hands, and avoiding contact with sick animals. Consideration should also be given to wearing protective gowns and gloves while handling animals, especially if camels have signs of upper respiratory tract disease [65].

7.9 Diagnostics

Diagnosis of MERS-CoV is a major concern in most diagnostic laboratories. Detecting the virus in respiratory tract samples remains the gold standard in diagnosing MERS-CoV infection. Several samples can be obtained from the respiratory system that can be used for diagnosing MERS-CoV infection. These include tracheal aspirates, nasopharyngeal swabs, and sputum. Tracheal aspirates and bronchoalveolar lavage samples (lower respiratory samples) yielded significantly higher viral copies than nasopharyngeal and sputum samples [27,28]. Lower respiratory tract specimens such as bronchoalveolar lavage fluid, sputum, and tracheal aspirates contain the highest viral loads (VLs) [27,28,66,67]. They should be collected whenever possible.

MERS can be confirmed by detection of viral nucleic acid or by serology. Analyzing whole blood and plasma also yielded a positive viral genome [66]. Real-time polymerase chain reaction (RT-PCR) is the mainstay for the diagnosis of MERS-CoV. The presence of viral nucleic acid can be confirmed by positive real-time reverse transcription PCR [32]. RT-PCR has limitations, including a long turnaround time and lack of common measurements and correlations with VL. It is recommended to screen for MERS-CoV using RT-PCR of the upstream of envelope gene (upE) followed by confirmation of the presence of one of the following genes; open reading frame 1A, 1B genes, or nucleocapsid (N) gene. Scientists are looking to implement viral sequencing on all negative samples by RT-PCR and they believe that can be exposed to another level of testing using sequencing of the RdRp gene or N gene and in this case, a positive result is diagnostic. It is also very important to maintain a continuous and random sequencing for MERS-CoV samples to be able to pick early viral mutations [68].

7.10 Vaccines

In vaccine production, a major limiting factor in designing comprehensive delivery systems for aerosol transmissible diseases is the enhancement of efficacy and easy vaccine administration [69,70]. Although monoclonal antibodies show promising antiviral effects in cell culture and animal models against MERS-CoV infection, their roles are still limited in large-scale disease prevention [39]. Vaccines still remain the best choice for MERS-CoV prevention. Vaccines against MERS-CoV thus far developed in the laboratory can be categorized as those based on viral vectors, such as adenovirus and Modified Vaccinia virus Ankara (MVA), or those based on recombinant viral proteins, DNAs, nanoparticles, and recombinant virus (Du et al., 2015). Two vaccine candidates, GLS-5300 and MERS001, have entered human clinical trials. The vaccine GLS-5300 was the first to be tested in healthy human volunteers. It is a DNA plasmid encoding the MERS-CoV S glycoprotein, requiring two to three injections delivered by electroporation [72].

The phase I clinical trial was started in 2016 at the Walter Reed Army Institute, and another phase I/II clinical trial is being conducted in Korea to test dosage safety and immunogenicity. Another vaccine candidate, MERS001, is a

replication-deficient chimpanzee adenovirus (ChAdOx1) containing the MERS-CoV S glycoprotein antigen [73,74]. This vaccine only requires one-time administration of $5 \times 10^9 - 5 \times 10^{10}$ virus particles via intramuscular route, and the local adverse events, as well as immunogenicity, will be evaluated in phase I clinical trial conducted at the University of Oxford. In addition, one more candidate vaccine has been tested in dromedary camels either for potential human use or straight into veterinary use. It explores a MVA as a vector to express MERS-CoV S glycoprotein [75]. The regimen involves immunization through intranasal as well as intramuscular routes twice at a 4-week interval. The vaccinated camels demonstrated a significant reduction of excreted infectious virus and viral RNA transcripts in vaccinated animals upon the MERS-CoV challenge.

Protection against MERS-CoV infection correlated with the presence of serum neutralizing antibodies to MERS-CoV. The remaining vaccine candidates are all in the stages of preclinical or laboratory development and invariably target the S glycoprotein or RBD critical for viral entry [39]. High levels of an immunologically active drug may lead to inflammatory and excessive immunological responses. Therefore safety precautions and new formulations may be needed to reduce the side effects [35]. Novavax, on June 6, 2013, announced that it has successfully produced a vaccine candidate designed to provide protection against the recently emerging MERS-CoV. The vaccine candidate was made using Novavax nanoparticle vaccine technology and is based on the major surface spike (S) protein.

7.11 Treatment strategies

The latency period of MERS-CoV is known to be between 2 and 14 days (median 5.4 days). From the development of the disease to the patient's admission, it takes 4 days and the period that people die from the disease takes 11.5 days [76,77]. In the first stage, flu-like symptoms such as fever, coughing, chilling, myalgia, and arthralgia are observed. After this, the respiratory difficulty is added. This quickly progresses to pneumonia [14]. A part (30%) of the patients complains of bowel symptoms like vomiting and diarrhea [78]. In the absence of specific antiviral therapy and lack of knowledge of viral kinetics, clinical management of MERS largely depends on supportive treatment and prevention of complications. Lung-protective ventilatory strategies for ARDS, inotropic support, antimicrobial therapy for co-infections, and renal replacement therapy for acute renal failure have been used [10,66,76,77]. Some patients with severe disease have been treated with systemic corticosteroids [79].

Several agents have shown inhibitory effects against MERS-CoV in cell cultures, including IFNs, ribavirin, cyclosporin A, and mycophenolic acid [46,47,80–82]. A combination of IFNa2b and ribavirin appears to have beneficial effects in reducing lung injury and inflammation when given to rhesus macaques within 8 h of inoculation with MERS-CoV [46,47]. This treatment combination was given to several severely ill patients with unfortunately fatal outcomes, likely because of late administration [83]. Currently, there is insufficient clinical data supporting the routine use of these agents, and randomized controlled trials are needed if supported by favorable responses in animal models. Convalescent plasma to be donated by patients who have fully recovered from MERS-CoV infection would be a good treatment option [78]. Several drugs inhibit MERS-CoV in cell culture, including ciclosporin and mycophenolic acid [80,81]. MERS-CoV-specific peptide fusion inhibitors, which function similarly to the HIV drug enfuvirtide, diminish virus replication in cultured cells, providing a novel approach to MERS treatment [84].

7.12 Clinical management approaches

7.12.1 Prevention and control of MERS

A high degree of awareness of the possibility of MERS-CoV infection and early isolation of suspected or confirmed MERS cases with proactive surveillance are crucial to preventing outbreaks. To decrease MERS-CoV human-to-human transmission and environmental contamination, aerosol-generating procedures (AGPs) should be avoided in crowded hospital accident and emergency departments and in inpatient medical wards without adequate infection control measures [85]. Droplet precautions are required for managing patients with confirmed MERS-CoV infection. Wearing a surgical mask within 1–2 m of the patient, and wearing a gown, gloves, mask, and eye protection on entering the patient's room, and removing them upon leaving, are important infection control measures. Airborne precautions should be applied for AGPs such as open suctioning or aspiration of the respiratory tract, intubation, bronchoscopy, or cardiopulmonary resuscitation. These precautions include wearing a half-mask air-purifying respirator, such as a United States National Institute for Occupational Safety and Health approved N95 filtering face-piece respirator or a European Standard-approved FFP2 or FFP3 filtering face-piece respirator. The respirator should fit properly, and all healthcare

workers should undergo in-depth training for the proper use, donning and doffing of the respirator, and performing a user seal check every time the respirator is used.

Cleaning environmental surfaces with water and detergent and applying commonly used disinfectants (such as hypochlorite) is an effective and sufficient procedure [7,8]. Unprotected or inadvertent exposure of healthcare workers to patients with MERS-CoV should prompt rapid quarantine. When MERS-CoV cases are suspected or diagnosed in the community and households, educational awareness of MERS-CoV and MERS prevention measures within the home could reduce further transmission and prevent outbreaks of community clusters. People with comorbidities such as diabetes, kidney disease, chronic lung disease, and cancer, or individuals on immunosuppressive treatments are at high risk of developing severe MERS-CoV disease, thus they should avoid close contact with camels and bats. Early recognition of MERS-CoV infections, improved compliance with internationally recommended infection control protocols, and rapid implementation of infection control measures are required to prevent outbreaks of MERS-CoV associated with health care facilities [86].

7.13 Summary and future prospective

With the advent of modern techniques in virology, immunology, and vaccinology, we have gained substantial insights into the biology of MERS-CoV, and its pathogenesis with unprecedented speed and accuracy. We are facing a difficult predicament when it comes to public health challenges in the new era of emerging and reemerging infectious diseases. On the one hand, the human population is becoming ever mobile and exposed to an increasing number of pathogens. On the other hand, translating basic discoveries into preventative and treatment applications has been exceedingly slow. MERS-CoV is a pathogen with epidemic potential that continues to cause sporadic human disease and remains on the WHO Blueprint 2020 priority list [87]. MERS-CoV endemic and at-risk countries must invest more in surveillance, public health research, and medical interventions. Early recognition of cases, improved compliance with internationally recommended infection control protocols, and rapid implementation of infection control measures are required to prevent health care facility−associated outbreaks of MERS-CoV.

References

[1] WHO Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Available from: http://www.who.int/emergencies/mers-cov/en/. [Accessed 23 May 2016].

[2] Chafekar A, Fielding BC. MERS-CoV: understanding the latest human coronavirus threat. Viruses 2018;10(2):93.

[3] Lee J. Better understanding of MERS coronavirus outbreak in Korea. J Korean Med Sci 2015;30(7):835−6.

[4] Al-Omari A, Rabaan AA, Salih S, Al-Tawfiq JA, Memish ZA. MERS coronavirus outbreak: implications for emerging viral infections. Diagn Microbiol Infect Dis 2019;93(3):265−85.

[5] WHO. 2022. Available from: https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers#tab = tab_1.

[6] Mubarak A, Alturaiki W, Hemida MG. Middle East respiratory syndrome coronavirus (MERS-CoV): infection, immunological response, and vaccine development. J Immunol Res 2019;2019:6491738.

[7] World Health Organization. Countries agree next steps to combat global health threat by MERS-CoV. Geneva, Switzerland: WHO; 2019.

[8] World Health Organization. Infection prevention and control during health care for probable or confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection: interim guidance: updated October 2019 (No. WHO/MERS/IPC/15.1 Rev. 1). World Health Organization; 2019b.

[9] Memish ZA, Perlman S, Van Kerkhove MD, Zumla A. Middle East respiratory syndrome. Lancet 2020;395(10229):1063−77.

[10] Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012;367(19):1814−20.

[11] Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev 2015;28(2):465−522.

[12] Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380(9859):2095−128.

[13] Mackay IM, Arden KE. Middle East respiratory syndrome: an emerging coronavirus infection tracked by the crowd. Virus Res 2015;202:60−88.

[14] Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis 2014;14(2):140−5.

[15] Azhar EI, Hui DS, Memish ZA, Drosten C, Zumla A. The middle east respiratory syndrome (MERS). Infect Dis Clin 2019;33(4):891−905.

[16] Al-Tawfiq JA, Auwaerter PG. Healthcare-associated infections: the hallmark of Middle East respiratory syndrome coronavirus with review of the literature. J Hospital Infect 2019;101(1):20−9.

[17] Zhao J, Alshukairi AN, Baharoon SA, Ahmed WA, Bokhari AA, Nehdi AM, et al. Recovery from the Middle East respiratory syndrome is associated with antibody and T cell responses. Sci Immunol 2017;2(14):eaan5393.
[18] Saad M, Omrani AS, Baig K, Bahloul A, Elzein F, Matin MA, et al. Clinical aspects and outcomes of 70 patients with Middle East respiratory syndrome coronavirus infection: a single-center experience in Saudi Arabia. Int J Infect Dis 2014;29:301–6.
[19] Cotten M, Watson SJ, Kellam P, Al-Rabeeah AA, Makhdoom HQ, Assiri A, et al. Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study. Lancet 2013;382(9909):1993–2002.
[20] Drosten C, Seilmaier M, Corman VM, Hartmann W, Scheible G, Sack S, et al. Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection. Lancet Infect Dis 2013;13(9):745–51.
[21] WHO. MERS Global summary and assessment of risk; 2019. Available at: https://www.who.int/csr/disease/coronavirus_infections/risk-assessment-august-2018.pdf. [Accessed 21 January 2019.
[22] Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, de Wit E, et al. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. MBio 2014;5(2):e00884-14.
[23] Hemida MG, Perera RA, Al Jassim RA, Kayali G, Siu LY, Wang P, et al. Seroepidemiology of Middle East respiratory syndrome (MERS) coronavirus in Saudi Arabia (1993) and Australia (2014) and characterisation of assay specificity. Eurosurveillance 2014;19(23):20828.
[24] Reusken CB, Farag EA, Jonges M, Godeke GJ, El-Sayed AM, Pas SD, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014. Eurosurveillance 2014;19(23):20829.
[25] Nowotny N, Kolodziejek J. Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013. Eurosurveillance 2014;19(16):20781.
[26] Hemida MG, Chu DK, Poon LL, Perera RA, Alhammadi MA, Ng HY, et al. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis 2014;20(7):1231.
[27] Memish ZA, Al-Tawfiq JA, Makhdoom HQ, Assiri A, Alhakeem RF, Albarrak A, et al. Respiratory tract samples, viral load, and genome fraction yield in patients with Middle East respiratory syndrome. J Infect Dis 2014;210(10):1590–4.
[28] Memish ZA, Cotten M, Meyer B, Watson SJ, Alsahafi AJ, Al Rabeeah AA, et al. Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. Emerg Infect Dis 2014;20(6):1012.
[29] Chu DK, Poon LL, Gomaa MM, Shehata MM, Perera RA, Zeid DA, et al.). MERS coronaviruses in dromedary camels, Egypt. Emerg Infect Dis 2014;20(6):1049.
[30] Reeves T, Samy AM, Peterson AT. MERS-CoV geography and ecology in the Middle East: analyses of reported camel exposures and a preliminary risk map. BMC Res Notes 2015;8(1):1–7.
[31] Khalafalla AI, Lu X, Al-Mubarak AI, Dalab AHS, Al-Busadah KA, Erdman DD. MERS-CoV in the upper respiratory tract and lungs of dromedary camels, Saudi Arabia, 2013–2014. Emerg Infect Dis 2015;21(7):1153.
[32] Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. Lancet 2015;386(9997):995–1007.
[33] Buzon MJ, Seiss K, Weiss R, Brass AL, Rosenberg ES, Pereyra F, et al.). Inhibition of HIV-1 integration in ex vivo-infected CD4 T cells from elite controllers. J Virol 2011;85(18):9646–50.
[34] Van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, et al. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. MBio 2012;3(6):e00473-12.
[35] Kato T, Takami Y, Deo VK, Park EY. Preparation of virus-like particle mimetic nanovesicles displaying the S protein of Middle East respiratory syndrome coronavirus using insect cells. J Biotechnol 2019;306:177–84.
[36] Arabi YM, Balkhy HH, Hayden FG, Bouchama A, Luke T, Baillie JK, et al. Middle East respiratory syndrome. N Engl J Med 2017;376(6):584–94.
[37] Meyerholz DK, Lambertz AM, McCray Jr PB. Dipeptidyl peptidase 4 distribution in the human respiratory tract: implications for the Middle East respiratory syndrome. Am J Pathol 2016;186(1):78–86.
[38] Widagdo, W., Raj, V. S., Schipper, D., Kolijn, K., Van Leenders, G. J., Bosch, et al. Differential expression of the Middle East respiratory syndrome coronavirus receptor in the upper respiratory tracts of humans and dromedary camels. J Virol 2016;90(9):4838–4842.
[39] Xu J, Jia W, Wang P, Zhang S, Shi X, Wang X, et al. Antibodies and vaccines against Middle East respiratory syndrome coronavirus. Emerg Microbes Infect 2019;8(1):841–56.
[40] Lu G, Hu Y, Wang Q, Qi J, Gao F, Li Y, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature 2013;500(7461):227–31.
[41] Mou H, Raj VS, Van Kuppeveld FJ, Rottier PJ, Haagmans BL, Bosch BJ. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J Virol 2013;87(16):9379–83.
[42] Wang N, Shi X, Jiang L, Zhang S, Wang D, Tong P, et al. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Res 2013;23(8):986–93.
[43] Molaei S, Dadkhah M, Asghariazar V, Karami C, Safarzadeh E. The immune response and immune evasion characteristics in SARS-CoV, MERS-CoV, and SARS-CoV-2: vaccine design strategies. Int Immunopharmacol 2020;92:107051.
[44] Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat Commun 2019;10(1):1–9.
[45] Arabi YM, Harthi A, Hussein J, Bouchama A, Johani S, Hajeer AH, et al. Severe neurologic syndrome associated with Middle East respiratory syndrome corona virus (MERS-CoV). Infection 2015;43(4):495–501.

[46] Falzarano D, De Wit E, Martellaro C, Callison J, Munster VJ, Feldmann H. Inhibition of novel β coronavirus replication by a combination of interferon-α2b and ribavirin. Sci Rep 2013;3(1):1−6.
[47] Falzarano D, De Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, et al. Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV−infected rhesus macaques. Nat Med 2013;19(10):1313−17.
[48] Faure E, Poissy J, Goffard A, Fournier C, Kipnis E, Titecat M, et al. Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside? PLoS One 2014;9(2):e88716.
[49] Müller MA, Meyer B, Corman VM, Al-Masri M, Turkestani A, Ritz D, et al. Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study. Lancet Infect Dis 2015;15(5):559−64.
[50] Alraddadi BM, Watson JT, Almarashi A, Abedi GR, Turkistani A, Sadran M, et al. Risk factors for primary Middle East respiratory syndrome coronavirus illness in humans, Saudi Arabia, 2014. Emerg Infect Dis 2016;22(1):49.
[51] Chan RW, Hemida MG, Kayali G, Chu DK, Poon LL, Alnaeem A, et al. Tropism and replication of Middle East respiratory syndrome coronavirus from dromedary camels in the human respiratory tract: an in-vitro and ex-vivo study. Lancet Respir Med 2014;2(10):813−22.
[52] Alosaimi B, Hamed ME, Naeem A, Alsharef AA, AlQahtani SY, AlDosari KM, et al. MERS-CoV infection is associated with downregulation of genes encoding Th1 and Th2 cytokines/chemokines and elevated inflammatory innate immune response in the lower respiratory tract. Cytokine 2020;126:154895.
[53] Nakamura H, Yoshimura K, McElvaney NG, Crystal RG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. J Clin Invest 1992;89(5):1478−84.
[54] Braciale TJ, Hahn YS. Immunity to viruses. Immunol Rev 2013;255(1):5.
[55] Totura AL, Baric RS. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. Curr Opin Virol 2012;2(3):264−75.
[56] Lau SK, Lau CC, Chan KH, Li CP, Chen H, Jin DY, et al. Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment. J Gen Virol 2013;94(12):2679−90.
[57] Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol 2017;39(5):529−39.
[58] Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, et al. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 2019;11(1):59.
[59] Bradley BT, Bryan A. Emerging respiratory infections: the infectious disease pathology of SARS, MERS, pandemic influenza, and Legionella. Semin Diagn Pathol 2019;36(3):152−9.
[60] Algahtani H, Subahi A, Shirah B. Neurological complications of middle east respiratory syndrome coronavirus: a report of two cases and review of the literature. Case Rep Neurol Med 2016;2016:3502683.
[61] Verstrepen K, Baisier L, Cauwer HD. Neurological manifestations of COVID-19, SARS and MERS. Acta Neurol Belgica 2020. Available from: https://doi.org/10.1007/s13760-020-01412-4.
[62] Drosten C, Muth D, Corman VM, Hussain R, Al Masri M, HajOmar W, et al. An observational, laboratory-based study of outbreaks of middle East respiratory syndrome coronavirus in Jeddah and Riyadh, kingdom of Saudi Arabia, 2014. Clin Infect Dis 2015;60(3):369−77.
[63] World Health Organization. Infection prevention and control of epidemic-and pandemic-prone acute respiratory diseases in health care: WHO interim guidelines (No. WHO/CDS/EPR/2007.6). Geneva: World Health Organization; 2007.
[64] Van Doremalen N, Bushmaker T, Karesh WB, Munster VJ. Stability of Middle East respiratory syndrome coronavirus in milk. Emerg Infect Dis 2014;20(7):1263.
[65] WHO Update on MERS-CoV Transmission from Animals to Humans (2022), and Interim Recommendations for At-risk Groups; 2014. Available from: http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_RA_20140613.pdf?ua = 1 [Accessed 16 February 2015].
[66] Guery B, Poissy J, El Mansouf L, Séjourné C, Ettahar N, Lemaire XMERS-CoV Study Group. Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. Lancet 2013;381(9885):2265−72.
[67] Poissy J, Goffard A, Parmentier-Decrucq E, Favory R, Kauv M, Kipnis E, et al. Kinetics and pattern of viral excretion in biological specimens of two MERS-CoV cases. J Clin Virol 2014;61(2):275−8.
[68] Al Johani S, Hajeer AH. MERS-CoV diagnosis: an update. J Infect Public Health 2016;9(3):216−19.
[69] Kim YS, Son A, Kim J, Kwon SB, Kim MH, Kim P, et al. Chaperna-mediated assembly of ferritin-based Middle East respiratory syndrome-coronavirus nanoparticles. Front Immunol 2018;9:1093.
[70] Zhang RZ, Tsai SS, Chao HR, Shieh YC, Chuang KP. Innovation of a new virus-like nanoparticle vaccine system for the specific aerosol relative disease. Aerosol Air Qual Res 2016;16(10):2421−7.
[71] Du, L., Jiang, S. Middle East respiratory syndrome: current status and future prospects for vaccine development. Expert Opin Biol Ther 2015;15(11):1647−1651.
[72] Muthumani K, Falzarano D, Reuschel EL, Tingey C, Flingai S, Villarreal DO, et al. A synthetic consensus anti−spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates. Sci Transl Med 2015;7(301) 301ra132.
[73] Alharbi NK, Padron-Regalado E, Thompson CP, Kupke A, Wells D, Sloan MA, et al. ChAdOx1 and MVA based vaccine candidates against MERS-CoV elicit neutralising antibodies and cellular immune responses in mice. Vaccine 2017;35(30):3780−8.
[74] Munster VJ, Wells D, Lambe T, Wright D, Fischer RJ, Bushmaker T, et al. Protective efficacy of a novel simian adenovirus vaccine against lethal MERS-CoV challenge in a transgenic human DPP4 mouse model. NPJ Vaccines 2017;2:28. Available from: https://doi.org/10.1038/s41541-017-0029-1.

[75] Haagmans BL, Van Den Brand JM, Raj VS, Volz A, Wohlsein P, Smits SL, et al. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science 2016;351(6268):77–81.
[76] Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. Lancet Infect Dis 2013;13(9):752–61.
[77] Assiri A, McGeer A, Perl TM, Price CS, Al Rabeeah AA, Cummings DA, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. N Engl J Med 2013;369(5):407–16.
[78] Hui DS, Memish ZA, Zumla A. Severe acute respiratory syndrome versus the Middle East respiratory syndrome. Curr Opin Pulmonary Med 2014;20(3):233–41.
[79] WHO MERS-CoV Research Group. State of knowledge and data gaps of Middle East respiratory syndrome coronavirus (MERS-CoV) in humans. PLoS Curr 2013;5.
[80] Chan JF, Chan KH, Kao RY, To KK, Zheng BJ, Li CP, et al. Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. J Infect 2013;67(6):606–16.
[81] De Wilde AH, Raj VS, Oudshoorn D, Bestebroer TM, van Nieuwkoop S, Limpens RW, et al. MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-α treatment. J Gen Virol 2013;94(Pt 8):1749.
[82] Josset L, Menachery VD, Gralinski LE, Agnihothram S, Sova P, Carter VS, et al. Cell host response to infection with novel human coronavirus EMC predicts potential antivirals and important differences with SARS coronavirus. MBio 2013;4(3).
[83] Al-Tawfiq JA, Momattin H, Dib J, Memish ZA. Ribavirin and interferon therapy in patients infected with the Middle East respiratory syndrome coronavirus: an observational study. Int J Infect Dis 2014;20:42–6.
[84] Lu L, Liu Q, Zhu Y, Chan KH, Qin L, Li Y, et al. Structure-based discovery of Middle East respiratory syndrome coronavirus fusion inhibitor. Nat Commun 2014;5(1):1–12.
[85] Hui DS, Azhar EI, Kim YJ, Memish ZA, Oh MD, Zumla A. Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. Lancet Infect Dis 2018;18(8):e217–27.
[86] Zumla A, Hui DS. Infection control and MERS-CoV in health-care workers. Lancet 2014;383(9932):1869–71.
[87] World Health Organization. A research and development Blueprint for action to prevent epidemics; 2018. Available from: http://www.who.int/csr/research-and-development/en.

Chapter 1

Lessons learned from the first pandemic of the 21st century, global experience, recommendations, and future directions

Kandati Kusuma[1,2], Praveen Belagal[3], Buddolla Viswanath[1] and Divi Venkata Ramana Sai Gopal[3,4,5]

[1]Dr. Buddolla's Institute of Life Sciences, A Unit of Dr. Buddolla's Educational Society, Tirupati, India, [2]Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, India, [3]DST PURSE Centre, Sri Venkateswara University, Tirupati, India, [4]Department of Virology, Sri Venkateswara University, Tirupati, India, [5]Vice-chancellor of Cluster University, Kurnool, Andhra Pradesh, India

1.1 Introduction

Many infectious diseases have swept the world, taking the lives of millions of people. Viral outbreaks of varying frequencies and severities have caused panic and havoc across the globe throughout history [1]. The 21st century witnessed a few pathogenic and contagious virus outbreaks of zoonotic origin including severe acute respiratory syndrome coronavirus (SARS-CoV-1&2), Ebola virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and Nipah virus [1]. SARS is an airborne virus and can spread through small droplets of saliva in a similar way to the cold and influenza. It was the first severe and readily transmissible new disease to emerge in the 21st century and showed a sustained human−human transmission along the routes of international air travel [2]. The 2003 outbreak of SARS shocked the world as it spread swiftly from continent to continent, resulting in >8000 infections, a total of 916 deaths globally with ~10% mortality and affecting local and regional economies [3]. In November 2002 the first case of SARS occurred in Foshan, China, and in June 2012, the first case of MERS died at a hospital in Jeddah, Saudi Arabia [4]. In November 2002 unusual cases of atypical pneumonia of unknown cause occurred in Foshan City, Guangdong province, in China, where many health care workers were infected [5]. Three laboratories—one each in Hong Kong, Germany, and the Centres for Disease Control and Prevention (CDCs) in Atlanta, Georgia, United States—nearly simultaneously isolated an apparently new coronavirus as the causative pathogen of SARS [3]. The infection was brought to Hong Kong on February 21, 2003, by a physician who had looked after similar cases of atypical pneumonia in the mainland China, leading to outbreaks in Hong Kong. On March 15, 2003, WHO officially declared an epidemic and labeled it as a SARS (later referred as SARS-CoV) [6−8]. The SARS-CoV epidemic quickly spread to 29 countries, but the global public health, medical, and scientific communities were not adequately prepared for the emergency. Chains of human-to-human transmission occurred in Toronto, Canada, Hong Kong Special Administrative Region of China, Chinese Taipei, Singapore, and Hanoi, Vietnam. The duration of the SARS epidemic was short and WHO declared the end of the SARS epidemic in July 2003 with a total of 8096 SARS cases and 774 deaths reported across 29 countries and regions [4]. The scientific effort demonstrated unusual international cooperation and was in turn facilitated by electronic communication. Media coverage provided accurate worldwide pictures to augment scientific data. As of March 1, 2004, there were 1695 citations related to SARS seen in the Medline [9]. One reason why SARS-CoV-2 spread is evidently much wider compared to SARS is the rapid urbanization and world trade network resulting in increased international travel during the last two decades. Hence, the control measures applied at the time of SARS-CoV-2 are no longer adequate in the current days, and more vigorous actions are required to control SARS-CoV-2 [10]. Besides, the duration in the infectious period between patients infected with SARS and those infected with SARS-CoV-2 is not the same. While in the former case, viral shedding peaks only when the patient's illness is advanced and respiratory symptoms appear [10], for SARS-CoV-2, the transmission can occur in the early phase of the illness, when the patients are completely asymptomatic [11]. Hence, isolation after the onset of symptoms might be ineffective in preventing virus transmission

Pandemic Outbreaks in the 21st Century. DOI: https://doi.org/10.1016/B978-0-323-85662-1.00010-0

and this also makes temperature screening less effective [12]. In comparison to other betacoronaviridae members SARS-CoV-2 has been shown to have higher transmissibility and a wider population distribution [13]. Despite being highly infectious and having higher transmissibility (designated as Reproduction number; R0), the severity of SARS-CoV-2 is much lesser compared to SARS. Though SARS caused major disruptions to international air travel, and impact on the health services and merchandise in the affected countries by 2004, SARS cases were hardly reported anywhere in the world [4]. Hence, any attempts of developing vaccines were stopped. In September 2012 Saudi Arabia reported the first case of the MERS, which was caused by MERS-CoV, another type of betacoronavirus. MERS-CoV spread to 27 countries and caused 2519 infections and 866 deaths by January 2020, with a Coronary Flow Rate (CFR) of 34.4% [14]. Both SARS-CoV and SARS-CoV-2 are closely related and originated in bats, which most likely are serving as a reservoir host for these two viruses [15–18]. SARS-CoV causes atypical pneumonia that spreads rapidly throughout or parts of Asia, North America, and Europe (Sino Biological). SARS-CoV was produced by recombination within bats and then transmitted to palm civets or other mammals via fecal–oral transmission. When virus-infected civets were transported to the Guangdong market, the infection spread among the civets in the market where it has probably acquired further new mutations before transmission to humans [19]. The concern is magnified by rapid population growth in areas with weak health systems, urbanization, globalization, climate change, civil conflict, and the changing nature of pathogen transmission between human and animal populations [20]. Influenza, smallpox, measles, and yellow fever reverberated for centuries, causing a huge burden on economies. As successive epidemics have swept the world, the scientific community has quickly learned about the emergence and transmission of communicable diseases. Epidemics usually occur when health systems are unprepared. During an unexpected epidemic, health authorities engage in damage control, fear drives action, and the desire to understand the threat is greatest [21]. Hong Kong was among the first cities affected by SARS, and its health care community suffered greatly from the epidemic. Some lessons from their experiences included recognition of the value of real-time information in a rapidly progressing epidemic with a large number of cases and the need for frequent patient updates, challenges of national efforts to maintain entry and exit health screening among international travelers, and implementation of home quarantine as an effective tool to control SARS transmission [3]. A novel beta CoV (SARS-CoV) of lineage B was confirmed as the cause of the atypical pneumonia cases on March 22, 2003 [4,6]. Outbreaks of various zoonotic viruses occur as a result of the influence of several factors including human-to-human contact and animal interaction with severe environmental changes [1]. At the end of 2019, an outbreak of severe respiratory illness occurred in Wuhan City, China. SARS-CoV-2 is responsible for the outbreak of severe respiratory illness (COVID-19) and is still spreading rapidly throughout the world [22]. On January 9, 2020, the Chinese CDC declared the identification of a novel Coronavirus [23]. As of January 29, 2021, around 100,455,529 confirmed cases of COVID-19 worldwide including 2,166,440 deaths, reported to WHO (Fig. 1.1). As of March 31, 2020, the SARS-CoV-2 has infected over a million and has caused more than 50,000 deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). Infectious disease threats, the fear, and panic in the public that may accompany various economic and social risks. With respect to outbreaks and epidemics (whether naturally occurring or human-initiated), there are obvious costs to the health care system in terms of medical treatment and outbreak control [20]. The virus that causes COVID-19, known as SARS-CoV-2, was first identified in Wuhan, China, on 31 December 2019 with the presentation of symptoms of atypical pneumonia. This case was further confirmed to be caused by the novel coronavirus, SARS-CoV-2 [24]. There are also other types of human coronaviruses. Coronaviruses have been found in many different animal species including birds and mammals. SARS-CoV-2 is thought to have reached from animals to humans through close contact, butchering, or eating undercooked meat in parts of Southern

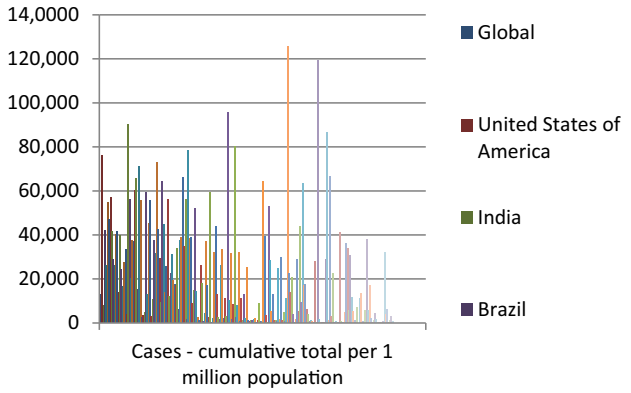

FIGURE 1.1 Covid-19 outbreaks across the world.

China. The most potential risk for the spread of COVID-19 worldwide is related to travel that is causing the regional and global spread of the disease (Bai et al., 2020). According to current observed epidemiologic characteristics, everyone is susceptible to Covid-19 and the median age is about 50 years [25–27].

1.2 First pandemic of the 21st century, severe acute respiratory syndrome

SARS is a SARS-associated coronavirus-caused viral respiratory disease. It was first observed at the end of February 2003 during an epidemic that started in China and spread to other nations. It is a member of the Coronaviridae family, which also encompasses many of the viruses that cause the common cold [9].

1.2.1 Structure of SARS-CoV

Coronavirus genomes are the largest among RNA viruses [28]. This family has been classified into at least three primary genera (alpha, beta, and gamma). Within this family, seven viruses are currently known to infect humans, namely, NL63 and 229E from the alpha genus and OC43, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2 from the beta genus. SARS-CoV has similar structural proteins as three previously known groups of coronaviruses: spike glycoprotein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) (Fig. 1.2). Coronavirus N protein is required for coronavirus RNA synthesis and has RNA chaperone activity that may be involved in template switch. SARS-CoV spike glycoprotein is 1255 amino acids long, with low (20–27%) amino acid similarity among other coronaviruses. Its carboxyl terminus (C-terminus) comprises the transmembrane region and the cytoplasmic tail (https://www.sinobiological.com/research/virus/sars-coronavirus-overview). In addition to the original genes, the SARS-CoV genome encodes another eight putative accessory proteins, known as ORFs 3a, 3b, 6, 7a, 7b, 8a, 8b, and 9b, which vary in length from 39 to 274 amino acids [30].

1.2.2 SARS-COV: mechanism of action

Coronaviruses appear as crown-like structures under electron microscope hence named coronavirus. They have positive-stranded RNA as their genomic material and have an outer envelope [24]. Coronaviruses belong to a family that comes under the order Nidovirales. Nidovirales order includes the viruses that use replication [23]. The life cycle of the virus with the host consists of the following five steps (Fig. 1.3): attachment, penetration, biosynthesis, maturation, and release. Once viruses bind to host receptors (attachment), they enter host cells through endocytosis or membrane fusion (penetration). Once viral contents are released inside the host cells, viral RNA enters the nucleus for replication. Viral mRNA is used to make viral proteins (biosynthesis). Then, new viral particles are made (maturation) and released. Coronaviruses consist of four structural proteins; spike (S), membrane (M), envelop (E), and nucleocapsid (N) [32]. Spike is composed of a transmembrane trimetric glycoprotein protruding from the viral surface, which determines the diversity of coronaviruses and host tropism. Spike comprises two functional subunits; S_1 subunit is responsible for binding to the host cell receptor and S_2 subunit is for the fusion of the viral and cellular membranes. Angiotensin-converting enzyme 2 (ACE2)

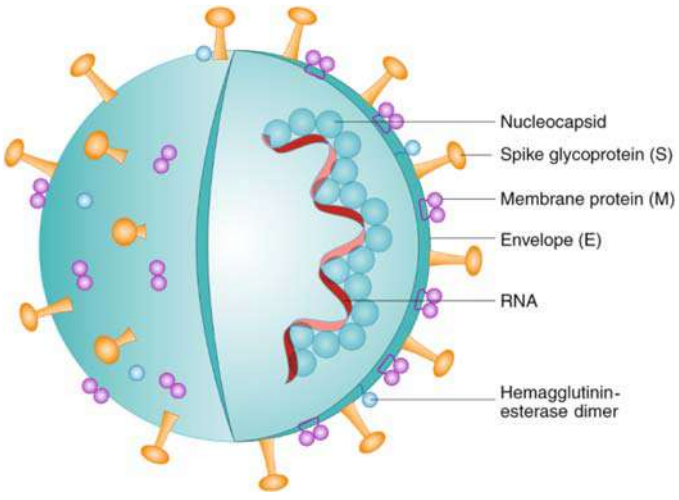

FIGURE 1.2 Representation of SARS-CoV structure [29]. *SARS-CoV*, Severe acute respiratory syndrome coronavirus.

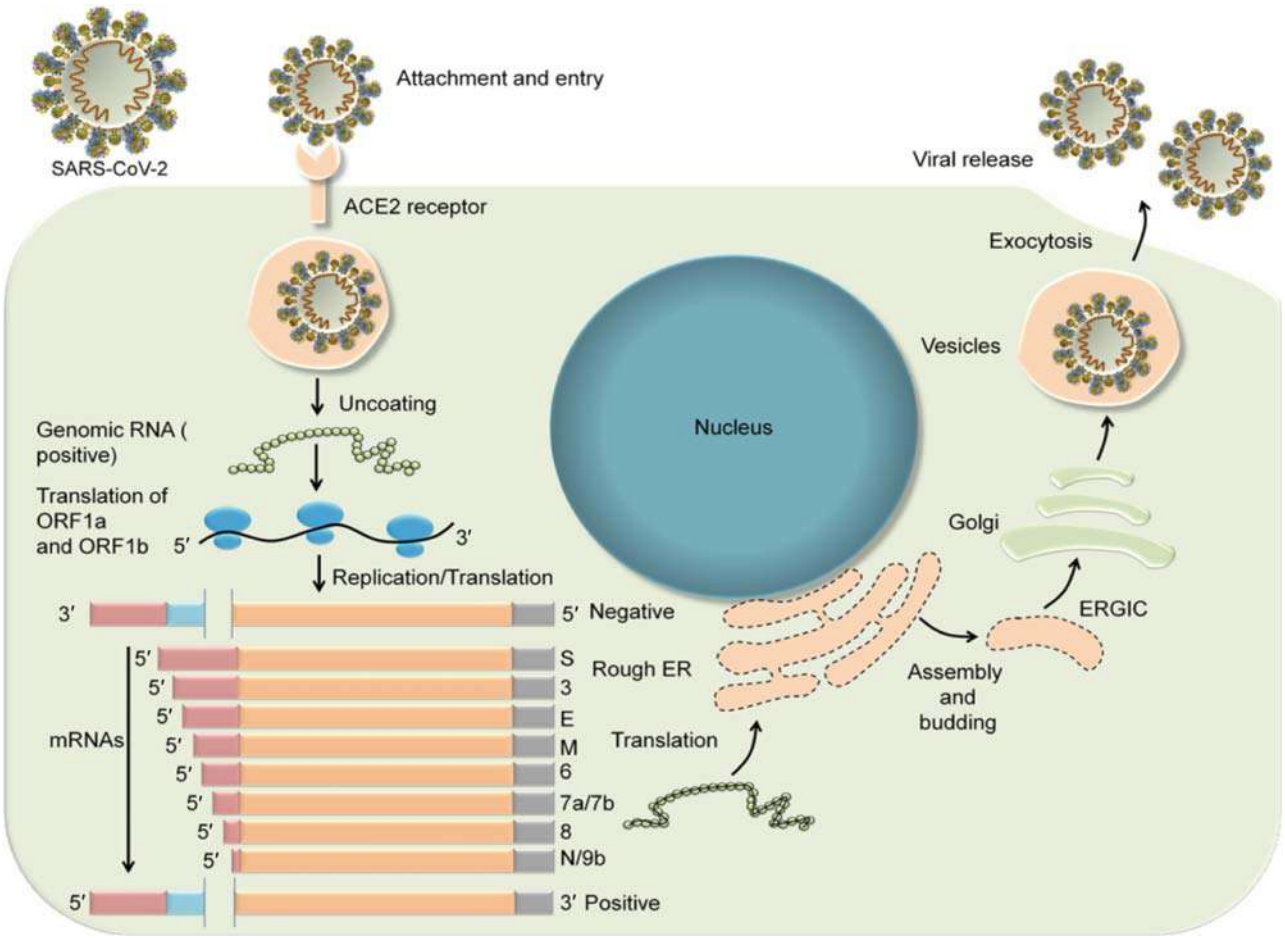

FIGURE 1.3 Mechanism of viral entry and replication and RNA packing in the human cell or invasion of SARS-CoV into the host cell [31]. *SARS-CoV*, Severe acute respiratory syndrome coronavirus.

was identified as a functional receptor for SARS-CoV [32]. The extracellular domain of the SARS-CoV spike glycoprotein consists of two heptad repeat regions that are known as heptad repeat region 1 and heptad repeat region 2. SARS-CoV spike glycoprotein has two functional domains: S1 and S2. S1 is responsible for the binding with its receptor ACE2 on host cells and defines the host range of the virus. S2 is the transmembrane subunit that facilitates viral and cellular membrane fusion. Membrane fusion occurs when there is a conformational change in the HRs to form a fusion core. The HRs of the protein fold into a coiled-coil structure—called the fusogenic state—causing the HR domains of the S protein to fold into a hairpin-like formation. This hairpin structure results in the cellular and viral membranes being pulled together and ultimately fusing. SARS-CoV infection can cause bronchial epithelial cell peeling, cilia damage, the formation of multinucleated giant cells, squamous cell aplasia, alveolar interstitial fiber cell hyperplasia, and fibrotic lung disease [33]. The SARS-CoV genome encodes 28 proteins in three distinct classes, many of them with unknown functions and sharing low similarity to other proteins. The structures of 16 SARS-CoV proteins or functional domains have been determined to date [34]. This virus was rapidly identified and characterized by a combination of classical virological methods and cutting-edge molecular biology. Electron microscopic examination of swabs and sputum specimens from affected patients revealed the presence of viral particles. Fortuitously, this newly identified agent replicated in Vero cells, in contrast to other human coronaviruses [9]. Moreover, cataloging the genome from human cases assisted in the search for the origin of this disease, when viruses related to the SARS-CoV were identified in animals [Himalayan palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*)] in a live animal market in Shenzhen, China [35]. Viral genomes from nasal swabs from palm civets were 99.8% homologous to the human SARS-CoV and represented a distinct phylogenetic group from the human isolates [4].

The virus can also spread through indirect contact transmission. Virus-containing droplets contaminate hands, people then contact the mucous membranes of the mouth, nose, and eyes, causing infection [36]. The genome of CoVs

(27−32 kb) is a single-stranded positive-sense RNA that is larger than any other RNA viruses [23]. The SARS-CoV-2 genome sequence shares ~80% sequence identity with SARS-CoV [18,37]. The transmission of SARS-CoV-2 is not limited to the respiratory tract [36]. Scientists aligned the full-length genome sequence of SARS-CoV-2 and other available genomes of betacoronaviruses. Results indicate the closest relationship of SARS-CoV-2 with the bat SARS-like coronavirus strain BatCov RaTG13, with an identity of 96%. These studies suggest that SARS-CoV-2 could be of bat origin, and SARS-CoV-2 might be naturally evolved from bat coronavirus RaTG13 [38]. Compared to SARS-CoV, many SARS-CoV-2 patients develop low levels of neutralizing antibodies and suffer prolonged illness [25]. Once the genome is released into the host cytosol, ORF1a and ORF1b are translated into viral replicase proteins, which are cleaved into individual Nsps (via host and viral proteases: PL^{pro}); these form the RNA-dependent RNA polymerase (Nsp12 derived from ORF1b) [39]. Here, the replicase components rearrange the endoplasmic reticulum into double-membrane vesicles that facilitate viral replication of genomic and subgenomic RNAs; the latter are translated into accessory and viral structural proteins that facilitate virus particle formation [40]. Epidemiological studies have shown that mortalities are higher in the elder population [18] and the incidence is much lower in children [41].

1.2.3 Global experiences

Members of a GOARN mission to China in late March warned that country's health authorities that if SARS was not brought under control in China, there would be no chance of controlling the global threat of SARS. Within days, the GOARN team announced that Chinese authorities had agreed to join the GOARN collaborative effort to contain the outbreak and prevent further international spread [42]. Although Chinese officials acknowledged that SARS had emerged in their country, they continued to downplay the extent and severity of the outbreak. This led the WHO team in Beijing to take the unusual measure of publicly expressing strong concern over inadequate reporting of SARS cases on April 16 [43]. The emergence of SARS in 2003 had a particularly devastating impact, both on human health and on the global economy, and demonstrated how rapidly viruses can spread around the world [34]. The outbreak also provided a stark warning of how ill-prepared we were at the time against a newly emerging infectious disease such as SARS [44]. The paucity of available scientific data for coronaviruses was a considerable disadvantage, but scientists mounted a rapid international response to the threat of SARS [45]. For instance, the SARS coronavirus was quickly identified and its genome was sequenced within weeks [6,46]. Now COVID-19 pandemic is a major health crisis and the world is experiencing an unprecedented challenge due to the coronavirus disease (COVID-19) pandemic [47]. However, the emerging global health system has made significant contributions to the protection and promotion of human health. According to UNESCO's monitoring, more than 160 countries implemented nationwide closures, which impacted over 87% of the world's student population [48].

However, successful public health prevention strategies eventually brought the SARS disease under control. Since then, researchers from all over the world have worked hard to learn more about the virus origins, inner workings, and interactions with host cells [34]. At the end of the SARS outbreak, the cases of over 1700 health care workers who had been affected were reported to the WHO, from China (19% of total cases), Canada (43%), France (29%), and Hong Kong (22%). During this epidemic, insufficient or inappropriate infection control measures, such as inconsistent use of personal protective equipment, reuse of N95 masks, and lack of adequate infection control, were related to the high risk of infection among health care workers [49]. The relative effectiveness of various strategies applied to SARS containment—the use of standardized case definitions and laboratory testing to identify the infected, the isolation of ill persons, and the quarantine of contacts—remains to be determined. Based on current knowledge, asymptomatic infected people only transmit SARS at a low rate. Thus in 2004 after the epidemic was contained, the WHO released a framework that was prepared according to the six phases of an epidemic, moving from preparedness, planning, and routine surveillance for cases, through to the prevention of the consequent international spread, to the disruption of global transmission [50]. The WHO, in collaboration with the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), and national governments, have been working with health care workers and scientists in affected countries to gather and share scientific evidence based on the previous coronavirus epidemic. This information gathering process has been beneficial for a better understanding of the virus and the disease it causes and for the regulation of outbreak response priorities, treatment approaches, and clinical management tactics [49]. When WHO declared on July 5 that all chains of SARS transmission had been broken, the disease was thought to have spread to more than 30 countries, only eight of which—Canada, China, Hong Kong, the Philippines, Singapore, Taiwan, the United States, and Vietnam—reported more than 10 probable cases [51].

In the case of Covid-19, global lockdowns helped respective countries to flatten the curve so that health care systems get ready with planning and infrastructure. In a highly populated country like India early lockdown largely slowed

down the infections. One best example is New Zealand which implemented a scientific approach [52] to successfully contain the Covid-19. On the contrary, many developed countries equipped with the preparedness and infrastructure, like United Kingdom, Italy, Spain, France, and the United States could not contain the spread effectively in the first wave of the pandemic. This could be largely due to an unscientific approach, lack of leadership, wrong decisions on prophylaxis, and so on. Since the 1918 pandemic, we have not improved much except in having ventilators, improved ICU facilities, blood thinners, few repurposed drugs, antigen/antibody testing, and so on. Yet, the three major nonmedical interventions such as masks, physical distancing, and hand washing were largely helpful during Covid-19. As a consequence, during the lockdowns for 4−6 months, the Covid-19 spread was relatively slowed down but the seasonal influenzas disappeared across continents as R0 of seasonal influenzas is less than that of Covid-19. No single intervention may be efficient to control pandemics like Covid-19.

Although accumulated knowledge and risk preparedness from the SARS/MERS epidemics allowed researchers to examine the effectiveness of strategic plans in dealing with the ongoing pandemic of COVID-19, several challenges have been raised in preventing the spread of COVID-19, such as the lack of medical supplies and laboratory facilities for the assessment of the disease and the presentation of a high number of asymptomatic cases [30]. The WHO Health Cluster framework is a gateway to useful resources to support COVID-19 preparedness and response [53]. Generally, each pandemic/epidemic has presented a public health emergency of uncertain scope and effect; thus essential elements of current approaches to pandemic preparedness and extenuation, such as the development of vaccines and stockpiling of antiviral drugs, necessitate detailed virological and immunological data on viruses with apparent pandemic potential. However, the development of vaccines against new strains is challenging. Therefore physicians and health workers have found themselves facing the massive challenge of preventing infections or stabilizing patients' conditions [30]. Several other countries implemented localized school closures; should these closures become nationwide, millions of additional learners will experience education disruption [48]. The real danger, however, may be in the long-term effects of the epidemic. Years of budget cuts and failure to meet students' basic needs make higher education especially vulnerable and potentially unequipped to handle a crisis like this [54].

1.3 Future directions

Advancements in science and technology related to diagnostic capacities, vaccines, and antiviral have provided more effective tools for preventing and responding to infectious disease threats. Effective preventive measures must be implemented to control it from global spreading. In addition, great effort should be made on the development of vaccines and antiviral drugs [55]. The use of a systems biology approach may enable us to finally understand the intricate dynamics between the cell and virus proteome that eventually constitute a particular disease phenotype [56]. The integration of transcriptome data, proteomic profiles, and detailed interaction networks between viral and cellular proteins should provide a system view of the intricate communication networks regulating virus infection at the cellular level. Platform approaches for each of these key system components are available, providing high-throughput identification of the interaction networks and the impact on host expression [57]. Comparing the responses to infection in lungs from SARS-CoV, H5N1 influenza virus, Ebola virus, and respiratory syncytial virus-infected hosts can further our understanding of how each virus modulates the innate immune response. The use of a systems genetic approach may be our best chance at identifying the host genes responsible for coronavirus disease and age-related susceptibility phenotypes [56]. The onset of the SARS epidemic in different continents has led to the formation of a successful laboratory network to identify the molecular mechanisms underlying the SARS infection [58]. Next to the development of early diagnostic tests and effective treatment strategies, it is most important to orchestrate research activities that lead to the development of vaccines and antiviral agents, as there is no established therapy to date. Even now in a situation of only a handful of new cases, SARS remains a major global health hazard that may reappear [58]. In the future the development of multiplex tests that differentiate among the members of coronaviruses and also influenzas with one patient sample would reduce testing burdens.

The Ministry of Family Welfare of the Government of India has set forth guidance for fighting COVID-19. The strategy involves establishing three types of facilities: fever clinic/COVID care center, dedicated COVID health center, and dedicated COVID hospital [60]. Plasma technology is now being considered as an alternative. Blood is collected from a person who has recovered from COVID-19. Separation of serum and screening is done for virus-neutralizing antibodies 59,60. The COVID-19 pandemic can be viewed as a crisis opportunity for the development of India [60]. There is a great experience in every country as far as the fight against COVID-19 is concerned. A combined effort to collect all these experiences and lessons learned from almost 213 countries will be a resource for future preparedness in terms of medical infrastructure, handling of quarantine centers, COVID-19 care centers, medicines, personal care

equipment, movable infrastructure, masks for frontline workers and working people, guidelines for social distancing, doctors, nurses, Asha village health workers maintenance personnel and legislative support. The most important is people's participation in all health care programs and strict adherence to guidelines [60]. Furthermore, the timely reporting of cases, updates on clinical status and disposition of patients, the real-time analysis of data, and the appropriate dissemination of information are essential for outbreak-managing decisions [30].

1.4 Conclusion

Now the pandemic by COVID-19 is a live issue affecting people worldwide. Strategies for preventing and controlling pandemic/epidemic viruses can be improved by being well-prepared. Preparedness strategies, which primarily include the quarantine of infected persons, self-protection (wearing facemasks, using disinfectants, washing hands, and disinfecting surfaces with bleach or alcohols), and social distancing are all considered to be important for a comprehensive plan that can be tested and promoted by conducting exercises to engage the whole of society. Without fundamental therapeutic interventions, current management is to reduce the virus spread and provide supportive care for diseased patients. There is an urgent need to develop targeted therapies. Understanding the difference in pediatric and adult responses to this virus may help to direct immune-based therapeutics. The ultimate goal is to develop a resilient global health infrastructure. Besides acquiring treatments, vaccines, and other preventive medicine, bio-surveillance is critical to preventing disease emergence and to counteracting its spread. During disasters, there is the utilitarian goal of doing the most good for as many people as possible with minimal harm. Vaccination is one of the most effective public health interventions and innovative strategies for the research and development of vaccines. The lessons learned from COVID-19 are maintaining self-hygiene, using masks, maintaining physical distance, and keeping the surroundings clean. Preparedness plans are crucial to build frameworks for emergency response, thereby providing countries with the opportunity to plan, strategize and mobilize human and capital resources before a pandemic occurs. Adequate and thorough plans ensure that countries can respond immediately when a pandemic is declared.

References

[1] Soman Pillai V, Krishna G, Valiya Veettil M. Nipah virus: past outbreaks and future containment. Viruses 2020;12(4):465.
[2] WHO. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: interim guidance, 25 January 2020. World Health Organization; 2020 (No. WHO/nCoV/Clinical/2020.2).
[3] LeDuc JW, Barry MA. SARS, the first pandemic of the 21st century. Emerg Infect Dis 2004;10(11):e26.
[4] Hui DS, Zumla A. Severe acute respiratory syndrome: historical, epidemiologic, and clinical features. Infect Dis Clin 2019;33(4):869–89.
[5] Zhao Z, Zhang F, Xu M, Huang K, Zhong W, Cai W, et al. Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China. J Med Microbiol 2003;52(8):715–20.
[6] Peiris JSM, Lai ST, Poon LLM, Guan Y, Yam LYC, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003;361(9366):1319–25.
[7] Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, Chan-Yeung M, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003;348(20):1977–85.
[8] World Health Organization. Transmission of SARS-CoV-2: implications for infection prevention precautions: scientific brief, 09 July 2020. World Health Organization; 2020.
[9] Cherry JD, Krogstad P. SARS: the first pandemic of the 21st century. Pediatr Res 2004;56(1):1–5.
[10] Strunk T, Inder T, Wang X, Burgner D, Mallard C, Levy O. Infection-induced inflammation and cerebral injury in preterm infants. Lancet Infect Dis 2014;14(8):751–62.
[11] Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med 2020;382(10):970–1.
[12] Singanayagam A, Patel M, Charlett A, Bernal JL, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Eurosurveillance 2020;25(32):2001483.
[13] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents 2020;55(3):105924.
[14] Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. Lancet 2015;386(9997):995–1007.
[15] Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 2013;503(7477):535–8.
[16] Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, et al. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS Pathog 2017;13(11):e1006698.
[17] Yang J. Asymptotic behavior of solutions for competitive models with a free boundary. Discrete Contin Dyn Syst A 2015;35(7):3253.

[18] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579(7798):270–3.
[19] Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17(3):181–92.
[20] Bloom DE, Cadarette D. Infectious disease threats in the twenty-first century: strengthening the global response. Front Immunol 2019;10:549.
[21] Gonzalez JP, Souris M, Valdivia-Granda W. Global spread of hemorrhagic fever viruses: predicting pandemics. Hemorrhagic fever viruses. New York: Humana Press; 2018. p. 3–31.
[22] Chakraborty C, Sharma AR, Sharma G, Bhattacharya M, Lee SS. SARS-CoV-2 causing pneumonia-associated respiratory disorder (COVID-19): diagnostic and proposed therapeutic options. Eur Rev Med Pharmacol Sci 2020;24(7):4016–26.
[23] Wang Y, Wang Y, Chen Y, Qin Q. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. J Med Virol 2020;92(6):568–76.
[24] Rabaan AA, Al-Ahmed SH, Haque S, Sah R, Tiwari R, Malik YS, et al. SARS-CoV-2, SARS-CoV, and MERS-COV: a comparative overview. Infez Med 2020;28(2):174–84.
[25] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579(7798):265–9.
[26] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395(10223):497–506.
[27] Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 2020;63(3):457–60.
[28] Pellett PE, Mitra S, Holland TC. Basics of virology. Handb Clin Neurol 2014;123:45–66.
[29] Florindo HF, Kleiner R, Vaskovich-Koubi D, Acúrcio RC, Carreira B, Yeini E, et al. Immune-mediated approaches against COVID-19. Nat Nanotechnol 2020;15(8):630–45.
[30] Abdelrahman Z, Li M, Wang X. Comparative review of SARS-CoV-2, SARS-CoV, MERS-CoV, and influenza a respiratory virus. Front Immunol 2020;11:2309.
[31] Trends in Immunology. Available from: https://www.cell.com/trends/immunology/fulltext/S1471-4906(20)30233-7.
[32] Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: a review. Clin Immunol 2020;215:108427.
[33] Luk HK, Li X, Fung J, Lau SK, Woo PC. Molecular epidemiology, evolution and phylogeny of SARS coronavirus. Infect Genet Evol 2019;71:21–30.
[34] Bartlam M, Xu Y, Rao Z. Structural proteomics of the SARS coronavirus: a model response to emerging infectious diseases. J Struct Funct Genomics 2007;8(2):85–97.
[35] Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 2003;302(5643):276–8.
[36] Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. Eur Respir J 2020;55(5):2000524.
[37] Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395(10224):565–74.
[38] Zhang C, Zheng W, Huang X, Bell EW, Zhou X, Zhang Y. Protein structure and sequence reanalysis of 2019-nCoV genome refutes snakes as its intermediate host and the unique similarity between its spike protein insertions and HIV-1. J Proteome Res 2020;19(4):1351–60.
[39] Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 transmission and pathogenesis. Trends Immunol 2020;41(12):1100–15.
[40] Wu HY, Brian DA. Subgenomic messenger RNA amplification in coronaviruses. Proc Natl Acad Sci USA 2010;107(27):12257–62.
[41] Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. Lancet Infect Dis 2020;20(6):689–96.
[42] Oberholtzer K, Sivitz L, Mack A, Lemon S, Mahmoud A, Knobler S, editors. Learning from SARS: preparing for the next disease outbreak: workshop summary. National Academies Press; 2004.
[43] Syndrome SA. Status of the outbreak and lessons for the immediate future. Geneva: WHO; 2003.
[44] Drosten C, Günther S, Preiser W, Van Der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003;348(20):1967–76.
[45] Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003;348(20):1953–66.
[46] Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, Van Amerongen G, Van Riel D, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 2003;362(9380):263–70.
[47] Hu Z, Lin X, Kaminga AC, Xu H. Impact of the COVID-19 epidemic on lifestyle behaviors and their association with subjective well-being among the general population in mainland China: cross-sectional study. J Med Internet Res 2020;22(8):e21176.
[48] de Oliveira Araújo FJ, de Lima LSA, Cidade PIM, Nobre CB, Neto MLR. Impact of Sars-Cov-2 and its reverberation in global higher education and mental health. Psychiatry Res 2020;288:112977.
[49] Suwantarat N, Apisarnthanarak A. Risks to healthcare workers with emerging diseases: lessons from MERS-CoV, Ebola, SARS, and avian flu. Curr Opin Infect Dis 2015;28(4):349–61.
[50] WHO C. Emergencies preparedness, response. Chikungunya; 2018.

[51] Knobler S, Mahmoud A, Lemon S, Mack A, Sivitz L, Oberholtzer K. Workshop Summary. Learning from SARS: preparing for the next disease outbreak; 2004. 11.
[52] Baker MG, Wilson N, Anglemyer A. Successful elimination of Covid-19 transmission in New Zealand. N Engl J Med 2020;383(8):e56. Available from: https://doi.org/10.1056/NEJMc2025203.
[53] Peeri NC, Shrestha N, Rahman MS, Zaki R, Tan Z, Bibi S, et al. The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned? Int J Epidemiol 2020;49(3):717–26.
[54] Inside Higher Ed. 2020. Available from: https://www.insidehighered.com/views/2020/03/12/coronavirus-couldhave-long-term-impact-state-funding-universities-opinion.
[55] Zheng J. SARS-CoV-2: an emerging coronavirus that causes a global threat. Int J Biol Sci 2020;16(10):1678.
[56] Frieman M, Baric R. Mechanisms of severe acute respiratory syndrome pathogenesis and innate immunomodulation. Microbiol Mol Biol Rev 2008;72(4):672–85.
[57] Tan SL, Ganji G, Paeper B, Proll S, Katze MG. Systems biology and the host response to viral infection. Nat Biotechnol 2007;25(12):1383–9.
[58] Groneberg DA, Hilgenfeld R, Zabel P. Molecular mechanisms of severe acute respiratory syndrome (SARS). Respir Res 2005;6(1):1–16.
[59] Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci USA 2020;117(17):9490–6.
[60] Munnoli PM, Nabapure S, Yeshavanth G. Post-COVID-19 precautions based on lessons learned from past pandemics: a review. J Public Health (Bangkok) 2020;1–9.