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Percentage Gender Distribution of Beta-Lactamase Producing *Staphylococcus Aureus* from Wound Isolates in Federal Teaching Hospital Owerri

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Abstract

This study was carried out in order to determine the incidence and antibiotic resistance patterns of beta-lactamase producing *Staphylococcus aureus* from wound infections among patients in Federal Teaching Hospital, Owerri. A total of 120 wounds swabs were aseptically collected from the hospital patients using standard bacteriological methods. Both male and female patients had incidence of 23(26.74%) and 14(16.28%) respectively. In conclusion, based on the findings of this study, the males had higher incidence compared to their female counterparts.

Keywords*: Gender, Beta-Lactamase, Staphylococcus Aureus, Wound*

Introduction

The challenges posed by *Staphylococcus aureus* to human hosts clinically have been documented since the late 1950s and early 1960s, when the bacterium was found to cause considerable menace in nosocomial or hospital acquired infections as a pathogen of notoriety with humans subjected to varying traumas, morbidities and accompanying fatalities or mortalities.[1-3]

This organism, is naturally endowed with exoenzymes, beta-lactamases, and other virulent and pathogenic factors that empower it to resist antimicrobials administered to attack it either by topical disinfection of wounds to reduce contaminations or therapeutically to assist in its elimination and cause wound healing by having a bactericidal effect on the staphylococcal pathogen.[4-5]

This innate ability of *Staphylococcus aureus* to resist antimicrobial agents directed against it in human infections arises from the fact that the bacterial pathogen produces beta-lactamases or penicillinases that hydrolyse and inactivate penicillins and cephalosporins antibiotics used against the bacterium, and most *staphylococcal* strains capable of producing beta-lactamase enzymes belong to methicillin-resistant *Staphylococcus aureus* (MRSA) having multiple resistances to other antibiotics like macrolides, aminoglycosides thereby constituting a nuisance in traumatic and surgical wounds.[6-7]

Wounds arise from the damages to parts of the human body resulting from several cuts, bites, sharp objects, burns, gunshots, surgical procedures, bruises and other mechanisms that affect the intact

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skin.[8] The development of a wound arises from the multifactorial factors that are at work in the affected human host.

The aim was to determine the incidence and antibiotic resistance patterns of beta-lactamase producing *Staphylococcus aureus* from wound infection.

Materials and Methods

Study Area

The study was done in Federal Medical Centre, Owerri, Nigeria.

Study Design

The study adopted cross-sectional design.

Sample Size Determination

The sample size was calculated using,

$N=Z^2pq/d^2$ (Araoye, 2014)

Where:

N= Minimum sample size for a given survey.

Z= Standard normal usually set at 1.96 which corresponds to the 95% confidence level.

P=Proportion in the target population in the same environment. Using 10.1% from the study by (Falake *et al.*, 2017)

p= 0.101.

q= 1.0-0.107 = 0.899

d= Degree of accuracy desired, usually set at 0.05 (5%) substituting for values.

$N= (1.96)^2 (0.101)(0.899)/ (0.05)^2= 120$

Specimen Collection

Specimens were collected aseptically using sterile swab sticks after cleaning the surrounding wounds with cotton wool soaked in (70%) alcohol to reduce contamination of skin commensal flora and the specimens taken to the laboratory for the investigation of extended incidence of beta-lactamase producing *S. aureus* in the wounds amongst the hospital patients.

Inoculation of Isolation Media

These classified wound swab samples were inoculated onto Mannitol salt agar, Chocolate blood agar, SDA, Blood agar and MacConkey medium respectively after (24-48) hours of incubation at 37˚C.[9]

After inoculation of these classified samples on this solid media, direct gram staining was done respectively. Thus, the cultures on Chocolate blood agar media were inoculated in carbon-di-oxide enriched atmosphere using the candle jar and incubated at 37°C for (24-48) hours. The cultures on Mannitol salt agar media were incubated at 37°C for (24-48) hours for selectively and differentially recovering isolates of *Staphylococcus aureus*, which appears yellow on this culture media and coagulase – negative *Stapylococcci* remain the color of the agar (Red). The culture on sabouraud dextrose agar (SDA) media were incubated at 37˚C for (2 days to 5 days) for selective isolation of fungi, which appears cream to white colonies.

Gram Staining

Gram staining of the isolated colonies was done as described by Cheesbrough.[9]

Methods:

- Smears were made with the aid of wire loop. The loop was used to transfer a colony of bacteria onto a slide in normal saline.
- This was evenly spread to cover an area of about 15-20m diameter on the slide.
- The smear was fixed by gentle flaming.
- It was covered with crystal violet stain for 50 seconds and rapidly washed off with clean water,
- It was then covered with Lugol's iodine for 50 seconds and washed off with water.
- The smear was decolorized rapidly with acetone for few seconds and washed off immediately.
- It was then covered with neutral red stain for 1 minute and washed off.
- The back of the slide was cleansed and placed on the draining rack for the smear to air-dry.
- The smears were examined with the oil immersion objective. According to Cheesbrough[9], the following results were observed.

  Gram positive bacteria - Dark purple
  Yeast cells - Dark purple
  Gram negative bacteria - Pale to dark red
  Nuclei of pus cells - Red
  Epithelial cells - Pale red

The arrangement and distribution of the bacteria on the slides e.g. clusters, chains, pairs, singles etc. were used in colony identification. However, the morphological examination of bacteria for cell size, shape and arrangement, staining properties, growth characteristic may not produce distinct differences. Hence further tests such as the biochemical tests and other specific tests were carried out for definitive identification of colonies of bacteria.

Biochemical Tests

Coliform organism and pseudomonas species were subjected to various biochemical tests such as carbohydrate fermentation, indole production, citrate utilization, ability to produce urease, as well as oxidase test. Other test carried out were motility test, while *Candida albicans* was identified with germ tube test. Catalase and coagulase tests were carried out for *Staphylococcus* and Streptococcus organisms. The biochemical test carried out are described below;

Indole test

This test was carried out as described by Cheesbrough.[9]

Method:

Each colony of the organisms was inoculated into sterile peptone water (3ml) in bijou bottle. This was incubated at 37°C for 24 hours. 0.5ml of Kavoc's reagent was added and shaken gently. The mixture was then examined. Interpretation: Positive reaction showed a red layer at the top of the broth while negative reaction showed a pale-yellow layer at the top of the broth.

Motility test

The test was carried out as described by Cheesbrough [9]

- A small drop of bacterial suspension was placed on a slide and cover glass.

- This was examined for motile organisms, using the 10 x and 40x objectives.
- Positive (true) motility was distinguished from the on-the-spot vibratory movement (Brownian movement) which is shown by all microorganisms and particles when suspended in a fluid.

Citrate utilization test

- This test was carried out as described by Cheesbrough.[9] -Simmon's citrate agar slop was inoculated with a light suspension of the organism using a straight wire and then the butt was stabbed.
- This was incubated at 37°C for 24 hours.
- Positive result was shown by a bright blue colour.
- Negative result was indicated by no change in colour of medium (usually green).

Oxidase test

This test was done as described by Cheesbrough.[9]

- A piece of filter paper was moistened with freshly prepared oxidase reagent in a clean Petri dish.
- Using a piece of glass rod, a colony of the organism was smeared on the filter paper.
- The development of a blue-purple colour within a few seconds was observed for, this indicates a positive oxidase test if within 10 seconds.

Catalase test

The test was carried out as described by Cheesbrough.[9]

- 3ml of hydrogen peroxide solution was poured into a test tube.
- Several colonies of the organism were inoculated into it using a sterile wooden stick.
- Bubbling was observed for.

Coagulase test

The method of Cheesbrough[9], was adopted in carrying out this test. Both slide and tube coagulase tests were carried to demonstrate the presence of bound and free coagulase respectively.

Slide Coagulase Test:

- 2 drops of distilled water were placed on each slide.
- The test organism was emulsified in each of the drops to make two thick suspensions.
- A loopful of plasma was added to one of the suspensions, and mixed gently and clumping was observed for within 10 seconds.
- Any agglutination within 10 seconds indicated positive test and no agglutination indicated a negative test.

Tube Coagulase Test:

Colonies that tested negative to slide coagulase test were further tested by the tube test.

- 0.2ml of plasma was pipetted into 3 tubes, (one for test *organism*, one for positive control and the last for negative control).

- 0.8ml of the test broth culture was added to the 1st tube, and 0.8ml of the *S. aureus* broth was also added to the second tube, and 0.8ml of sterile broth to the last tube.
- They were gently mixed and incubated at 37°C for 1 hour at first and if no clotting, for further 3 hours and they were still negative for 24 hours.

Germ tube test

The method of Cheesbrough [9], was adopted in carrying out this test.

- The test *organism* was inoculated into 0.5ml of human serum and incubated at 37°C for 2-3 hours.
- Pasteur pipette was used to transfer a drop of the serum yeast culture to a glass slide, and covered with cover glass.
- This was examined microscopically using the 10x and 40x objectives.
- The production of sprouting yeast cells that is tube like outgrowth from the cells indicated a positive germ tube test.

Antimicrobial Sensitivity

The modified Kirby-Bauer Sensitivity testing techniques described by Cheesbrough [9] was adopted.

- The isolated colonies of *organism* were emulsified in 3-4ml of sterile physiological saline using wire loop.
- Using a good light, the turbidity of the suspension was matched with that of the standard.
- Using a sterile swab stick, the Mueller Hinton agar was inoculated, while the excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The swab was then streaked evenly over the surface of the medium in three directions, rotating the plate approximately 60^0 to ensure even distribution.
- With the Petri dish lid in place, the surface of the agar was left for 3-5 minutes to dry.
- Using sterile forceps, the antimicrobial discs were placed on the inoculated plate.
- Within 30 minutes of applying the discs, the plates were inverted and incubated aerobically at 37°C for 24 hours. After overnight incubation, the control and test plates were examined to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured.

Iodometric Method for Detection of Beta-Lactamase Production

All the isolates were tested for their ability to produce β-lactamase using the Perret's iodometric assay as modified by Workman and Farrar. Nutrient agar plates containing 0.2% starch were prepared and pure cultures of S. *aureus* strains were streaked on the agar surface and incubate at 37°C overnight. Each plate was flooded with 3ml of freshly prepared phosphate buffered saline (pH 6.4) containing iodine (3 mg/ml), potassium iodide (15 mg/ml) and penicillin G (50 mg/ml). The solution was poured away and the plates were left for 10 min. The decolourisation of the starch-iodine complex indicates β-lactamase production.

Results

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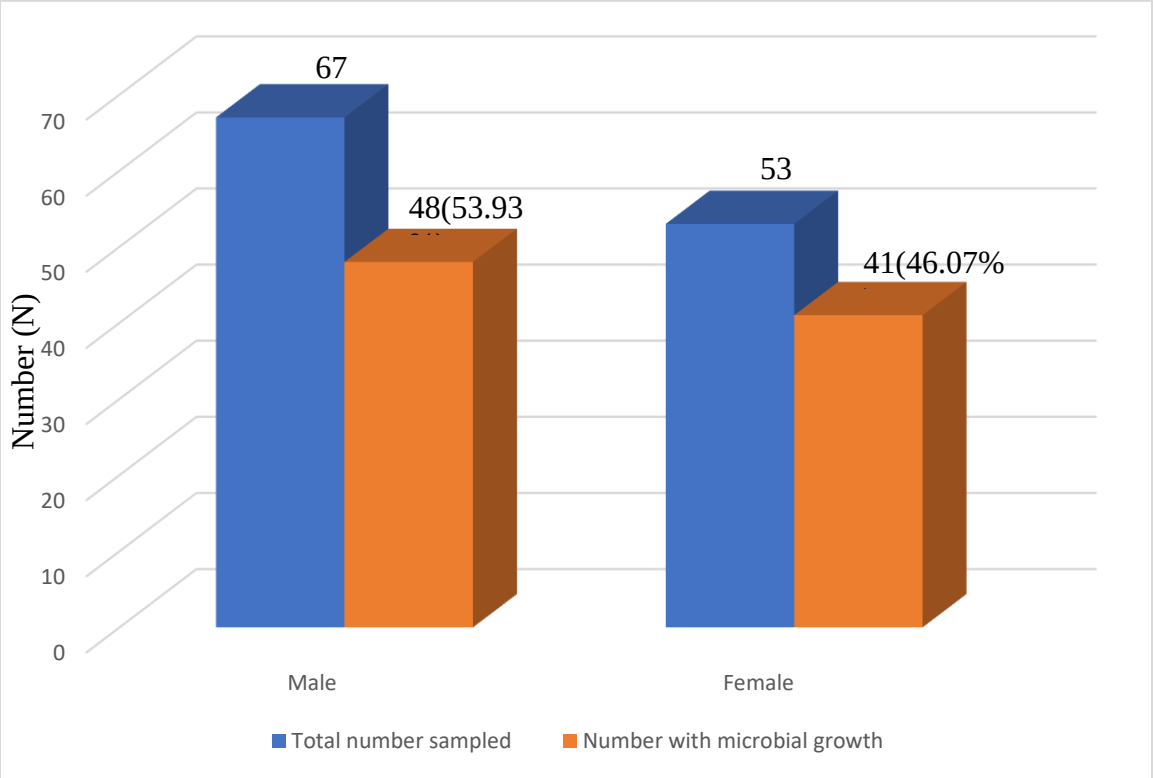
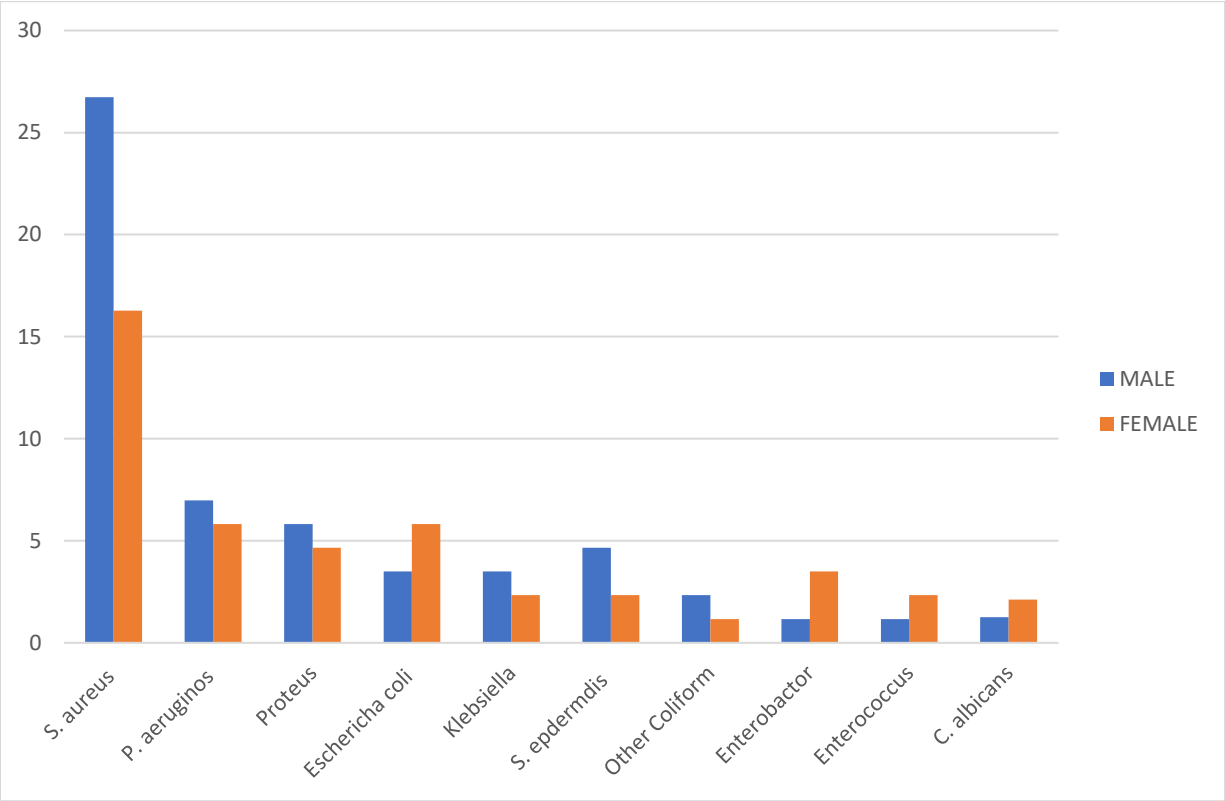


Chi-square = 0.8059, df = 1, P –value = 0.369*
Figure 1: Microbial growth of wounds by gender
* Not statistically significant



Chi-square = 5.8811, df = 1, P –value = 0.004*
Figure 2: Percentage Gender Distribution of Wound Isolates In Hospital Patients
* Not statistically significant

Discussion

In addition, *S. aureus* isolates (37), found in this study, were observed to be distributed in both male and female patient's wounds, though the males had higher incidence compared to their female counterparts of 23(26.74%) and 14(16.28%). The difference in the occurrence of S. *aureus* in both sexes could emanate from the fact that the organism *is a* normal microbiota of the human body along with bacteria encountered in this work. The bacterium S. *aureus* could be transferred into patients wounds from exogenous sources or endogenous routes especially in hospitalized patients.[10-11]

Also, the incidence of the bacterial isolates from wounds of hospital patients was more among the age groups of (42-51), (32-41), (22-31) followed by (12-21) and (52-61) with incidences of 35(28%), 27(21.6%), 21(16.8%), 16(12.8%), and 11(8.8%) respectively while age groups (62-71) and (2-11) had incidences of 8(6.4%) and 7(5.6%). These findings could be attributed possibly due to the increased economic and social activities of the actively engaged adult patients who expose themselves to various hazards leading to injuries compared to very young and children, as well as

the elderly adult patient with reduced vigour and retired from activities prone to high risk of acquiring injuries and accidents exposure to accidents, at work place and others needed for their survival in life.

Conclusion

In conclusion, based on the findings of this study, the males had higher incidence compared to their female counterparts.

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