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Antibiotic Susceptibility Pattern of Gram-Negative Bacteria Isolated from Infected Wound of Patients in Two Health-Care Centers in Gboko Town

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Abstract

The rising cases of multi-drug resistant bacteria have received considerable attention from patients and clinicians as a result of difficulties encountered during treatment. This has posed great challenges to pharmaceutical practitioners for continued search for effective antibiotics. The present study aimed at investigating antibiotic susceptibility profile of Gram-negative bacteria isolated from infected wound in two selected privately owned health-care centers in Gboko town. A total of thirty (30) wound samples, fifteen (15) from each of the hospital were collected using swab sticks and analyzed and the isolates tested for MDR using disc diffusion method. The result showed that the wounds were infected with *Escherichia coli* (46.7%), *Salmonella spp* (40%) and *Pseudomonas spp* (13.3%). The three isolates displayed varying degrees of resistance to antibiotics. *Escherichia coli* showed the highest resistance to streptomycin (92.8%), Augmentin (92.8%), Septrin (78%), Gentamycin (78.5%), Amoxacillin (71.4%) and Chloramphenicol (71.4%) and least resistance was observed with Pefloxacin (50%), Tarivid (50%), Sparfloxacin (57%), Ciprofloxacin (50%) antibiotics. *Salmonella spp* showed the highest resistance to Septrin (100%), Augmentin (91%), Streptomycin (91%), Amoxacillin (83.3%), Chloramphenicol (83.3%), Tarivid (83.3%) and least resistance to Sparfloxacin (33.3%), Ciprofloxacin (41.6%) and Pefloxacin (41.6%) antibiotics. *Pseudomonas spp* showed (100%) resistance to Gentamycin, Augmentin, Amoxacillin, Ciprofloxacin, Sparfloxacin, Septrin and Streptomycin, though the resistance was observed to be slightly minimal to Pefloxacin (75%), as well as Tarivid and Chloramphenicol. Antibiotic susceptibility test is recommended prior to administration of antibiotics for effective treatment and periodic monitoring is also advocated to check emerging MDR trends as a guide to health authorities.

Keywords: Antibiotics; Susceptibility; Resistance; MDR

Introduction

Wound is defined as an injury to any of the body tissues especially when it is caused by physical means that interrupts continuity [1]. Wounds can be classified as accidental, pathological or post-operative. Whatever the nature of the wound, infection is the attachment of microorganism to host cells and they proliferate, colonize and become better placed to cause damage to the host tissue [2].

The exposed subs-cutaneous tissue provides a favorable substratum for a wide variety of microorganisms to contaminate and colonize, and if the involved tissue is devitalized and the host immune response is compromised, the conditions become optional for microbial growth [3]. Wound provides a nutritive, moist and warm optimal environment for colonization, proliferation and infection by microbes. Different species of bacteria are found on human skin in the nasopharnyx, gastrointestinal tract and other parts of the body with little potential to cause disease, because of the first line of defense of the body. Despite the skin barrier, any breach in the skin surface whether trauma, accident, surgical operation or burn will open the door for bacterial infections [4].

Infected Wounds are wounds that are colonized with bacteria or other microorganisms that cause its deterioration and a delay in wound healing. Wounds are mostly contaminated by bacteria. Infected wounds result when immune defenses of the body are stunned or cannot withstand common bacterial growth. Wound infection caused by surgery is a severe health challenge. Previous studies have revealed that about 70 percent of the deaths of patients who have undertaken surgical operations are triggered by surgical spot infections [5].

Cases of infected wounds are mostly caused by bacteria, coining either from the skin and other portions of the body or the exterior environment. The skin comprises of bacteria (normal flora) which are generally innocuous and inoffensive provided the skin is unbroken. The protective barrier formed by the skin when it is disrupted, there is a wound, and these normal floras are able to inhabit the bruised area.

This leads to further tissue impairment and may delay wound healing by stimulating further soreness, which protracts the sequence of wound curing. Contamination from other parts of the body may also cause wound infection. Poor wound dressing techniques and unhygienic conditions may increase the risk for wound infection.

Wound infections is reportedly one of the most common acquired infections and are an important causes of morbidity that has accounted for 70% to 80% mortality. Different groups of microorganism like bacteria, fungi and protozoa have been isolated and implicated in diverse wound infections. The microorganisms can also exist in polymicrobial communities especially in the margin of wounds and in chronic wounds [6].

In a healthy individual, the internal tissues are usually free of microorganisms; however, the surface tissue in constant contact with the environment is colonized by a number of indigenous microbiota which is composed mainly of bacterial belonging to about 19 phyla. Some of these bacteria are capable of entering the blood through wounds which provides an optimum environment conducive for colonization [7]. The organisms which are regularly found on any wound are referred to as Indigenous microbiota, and are found on the epidermis and the upper portion of hair follicles. The total number of bacteria estimated to be on an average human is 10^{12}. These microbes are usually non-pathogenic

or in some cases beneficial. Such benefits include the prevention of colonization by pathogenic strains of bacteria on the surface of the skin either by competing for nutrients, secreting toxic chemical against them, or stimulating the skin immune system to respond to the threat [8].

An average of 5-6 strains of organisms is often implicated in the infection with a mixture of aerobic and anaerobic organisms. The most common isolated bacteria observed in previous studies include; *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Proteus spp*, coagulase-negative *Staphylococcus* and *Staphylococcus epidermidis* [9], *Pseudomonas aeruginosa* and bacteria belonging to family *Enterobacteriaceae* [10]. Wound infections have been a problem in the field of medicine for a long time. The presence of extraneousconstituents increases the possibility of severe infection even with prettyslight bacterial inoculums [6]. Other microbial isolates that have been incriminated in cases of wound infection include; *Streptococcus pyogenes, Staphylococcus aureus, Klebsiellaspp, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter and Enterobacter. Candida albicans, Streptococcus faecalis, and C. tropicalis* have also been implicated as etiological agents [11].

The selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the pathophysiology of the infectious and pharmaco-kinetics of the intended therapeutic agents. Hence the treatment of infections in patients becomes difficult. Studies are required to assess the right kind of antibiotics and the appropriate concentrations to be used in infections taking into consideration the etiology of the infection and duration of the antibiotic treatment [9].

Antibiotics are often prescribed for the adjunctive treatment of skin and wound infections. The choice of antibiotic is usually based on previously published susceptibility testing and previous clinical success. There is concern that bacteria have increased resistant to the currently prescribed antibiotics [12]. Susceptibility testing is the determination of the bacterial pattern of resistant to a number of antibiotics. It would be ideal if susceptibility testing could always be undertaken before the prescription of antibiotics. Unfortunately, it usually takes from several days to weeks to cultivate and do susceptibility tests on anaerobic bacteria.

Anaerobic bacteria are important because they dominate the diagnosed flora. They are commonly found in different infections. Some of these infections are serious and have high mortality rate. Isolation and identification of anaerobic bacteria can be complex, difficult, labor intensive and expensive. The majority of these infections have caused mixtures of numerous strains of aerobic and anaerobic bacteria. Interpreting culture to establish the extent to which any one particular anaerobic in the mixture is contributing to infection is difficult [13].

Advances in to control of infections have not completely eradicated this problem because of development of drug resistance. Over the last several decades, gram-negative organisms have emerged as the most common etiologic agents of invasive infection by virtue of their large repertoire of virulence factors and antimicrobial resistant traits. It is just not sufficient to be aware of the microorganism that pose a problem for wound patient, but to have an in-depth knowledge of the organism that are predominant in that particular period along with their sensitivity pattern. This would be crucial to reduce the overall infection related morbidity and mortality [14]. The Center for Disease Control and Prevention (CDC) reported that healthcare related infection showed that antimicrobial-resistant gram-negative bacilli are an emerging risk in the healthcare system [15].

Aim and Objectives of the Study

The work aimed to investigate the antibiotic susceptibility profile of Gram-negative bacteria isolated from infected wound in selected health-care centers in Gboko town.

The objectives of this research were:

ι. To isolate and characterize Gram-negative bacteria from infected wounds using conventional culture approaches.

ιι. To determine antibiotic susceptibility profile of Gram-negative bacteria from infected wound.

The study will enlighten the general public and the effects of misuse, mismanagement and continuous use of antibiotics and further educates the general public on the importance of antibiotic sensitivity testing so as to avoid drug resistance. It will provide a baseline data in the subject matter in this part of the world.

The research work covered investigation of antibiotic susceptibility pattern of gram-negative bacteria isolated from infected wound in some health- care centers in Gboko-town and is limited to Gram-negative bacteria isolated from infected wound to the exclusion of Gram-positive bacteria from wounds.

Materials and Methods

Study area

This research project work was carried out in Gboko metropolis of Benue State. Gboko local government area is among the largest and the most popular local government area in Benue state, with a population of about 500,00 people. It is located at latitude 18/30 and long 7/45. The majority of the populations are farmers with few civil servant and traders.

Sterilization of equipment and work environment

Glasswares were washed with detergent in tap water and sterilized by autoclaving at 121°C for 15 minutes. Swab sticks were brought sterile and used once for each specimen. The work-table surface was sterilized with 98% methanol.

Sample collection

Cross sectional study was conducted on patients with wound infections in selected health-care centers in Gboko town. Sterile swab sticks were used to swab the wound surface not without taking in to cognizance the duration of the patient has stayed in the hospital, the swab was replaced into its jacket. The swab sticks were accurately labeled and taken to the laboratory for analysis.

Preparation of culture media

Media for isolation of bacteria (Blood agar and Eosin methylene blue agar) were prepared according to manufacturer's specifications.

Isolation and identification of bacteria species

Wound swab samples were cultured on blood agar for aerobic plate count by streaking. The plates were incubated at 37°C for 24 hrs. Distinctive colonies on blood agar were sub- cultured on Eosin Methylene Blue agar (EMB) by streaking. The plates were incubated at 37°C for 24 hrs.

Biochemical tests

Indole, Methyl red, and Voges-Prokauer tests were carried out on the isolates for further characterization as described by Cheesbrough [16].

Gram staining

Isolates were also Gram stained to determine their shape and Gram-reactions as described by Cheesbrough [16].

Antibiotic susceptibility test

Kirby-Bauer disc diffusion method was adopted for susceptibility testing. Pure colonies of the isolates were cultured on Mueller-Hinton agar by spread plating. Antibiotic sensitivity discs were then placed on the surface of the culture using a sterile forceps. The plates were incubated at 37°C for 24 hrs. The zones of inhibition were measured using a meter rule [17].

Results

Antibiotic susceptibility pattern of Gram-negative bacteria isolated from infected wound in various health-care centers Gboko was investigated. Table 1 shows the biochemical test result of the isolated Gram negative bacteria from wounds samples obtained in the study. Three isolates were identified to include; *Escherichia coli, Salmonella* spp and *Pseudomonas* spp from wound samples obtained in Baki hospital Gboko. Table 2 shows that only *Escherichia coli* and *Salmonella* spp were isolated from Penuel orthopedic hospital, Gboko, Benue State.

Sample	CFU	Citrate	Indole	M.r	Gram-staining	Bacteria isolates
1	35	-	-	+	-	*Escherichia Coli*
2	2	+	-	-	-	*Salmonella Spp*
3	41	+	-	-	-	*Pseudomonas Spp*
4	15	-	-	+	-	*Escherichia Coli*
5	89	+	-	-	-	*Pseudomonas Spp*
6	101	+	-	-	-	*Salmonella Spp*
7	11	-	+	+	-	*Escherichia Coli*
8	121	-	-	-	-	*Escherichia Coli*
9	38	+	-	-	-	*Pseudomonas Spp*
10	21	+	-	-	-	*Salmonella Spp*
11	111	-	-	+	-	*Escherichia Coli*
12	8	-	+	+	-	*Escherichia Coli*
13	13	-	+	+	-	*Escherichia Coli*
14	105	+	-	-	-	*Salmonella Spp*
15	15	-	+	+	-	*Escherichia Coli*

Key: (-): Negative; MR: Methyl Red Test; (+): Positive; CFU: Colony Forming Units

Table 1: Gram-negative bacteria isolated from infected wounds of patients in Baki hospital, Gboko, Benue state, August, 2017.

Sample	CFU	Citrate	Indole	M.r	Gram-staining	Bacteria isolates
1	41	-	-	+	-	*Escherichia Coli*
2	30	-	+	+	-	*Escherichia Coli*
3	38	+	-	-	-	*Salmonella Spp*
4	56	-	+	+	-	*Escherichia Coli*
5	10	-	+	+	-	*Escherichia Coli*
6	62	-	-	+	-	*Escherichia Coli*
7	21	-	+	+	-	*Escherichia Coli*
8	70	+	-	-	-	*Salmonella Spp*
9	41	-	-	+	-	*Escherichia Coli*
10	11	+	-	-	-	*Salmonella Spp*
11	36	-	+	+	-	*Escherichia Coli*
12	61	+	-	-	-	*Salmonella Spp*
13	48	+	-	-	-	*Salmonella Spp*
14	56	+	-	-	-	*Salmonella Spp*
15	110	-	-	+	-	*Escherichia Coli*

Key: (-): Negative; MR: Methyl Red Test; (+): Positive; CFU: Colony Forming Units

Table 2: Gram-negative bacteria isolated from infected wounds of patients in Penuel orthopedic hospital, Gboko, Benue State, August, 2017.

S/no	Bacteria solates	Baki	Penuel	Total	Percentage (%)
1	*Escherichia Coli*	5	9	14	46.7 %
2	*Pseudomonas Spp*	4	0	4	13.3 %
3	*Salmonella Spp*	6	6	12	40 %

Table 3: Summary results of Gram-negative bacteria isolated from infected wound in two health-care centres, Gboko town, Benue State.
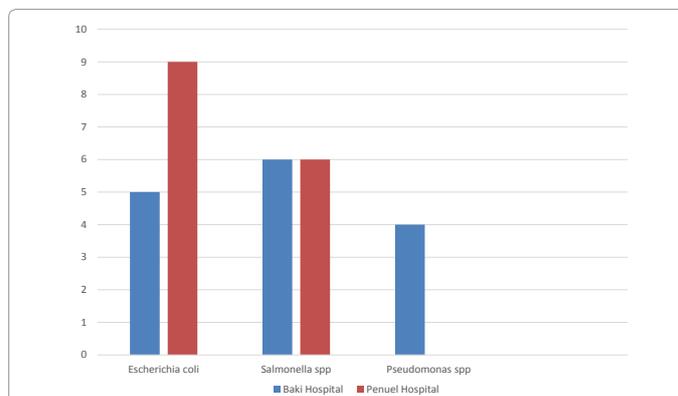

Figure 1: Bar chart showing the occurrence of gram-negative bacteria isolated from infected wound in two health-care centers in Gboko.

Antibiotics	*Escherichia coli* (n=14)	*Salmonella spp* (n=12)	*Pseudomonas spp* (n=4)
PEF	7(50%)	5(41.6%)	3(75%)
OFX	7(50%)	10(83.3%)	3(75%)
S	13(92.8%)	11(91.6%)	4(100%)
SXT	11(78.5%)	12(100%)	4(100%)
CH	10(71.4%)	10(83.3%)	3(75%)
SP	8(57.1%)	4(33.3%)	4(100%)
CPX	7(50%)	5(41.6%)	4(100%)
AM	10(71.4%)	10(83.3%)	4(100%)
AU	13(92.8%)	11(91.6%)	4(100%)
CN	11(78.5%)	8(66.6%)	4(100%)

Key: PEF: Pefloxacin; PFX: Tarivid; S: Streptomycin; SXT: Septrin; CH: Chloramphenicol; SP: Sparfloxacin; CPX: Ciprofloxacin; AM: Amoxacillin; AU: Augmentin; CN: Gentamycin

Table 4: Antibiotic resistance pattern of gram-negative bacteria isolates from infected wound in two health-care centres, Gboko.

Escherichia coli (46.7%) was the most frequent Gram negative bacteria isolated from the two hospitals studied while *Salmonella* spp (40%) was the next most frequent (Table 3) (Figure 1).

Table 4 shows antibiotic resistance pattern of gram-negative bacteria isolates from infected wound in two health-care centres, Gboko. *Escherichia coli* showed the highest resistance to streptomycin (92.8%), Augmentin (92.8%), Septrin (78.5%), Gentamycin (78.5%), Amoxacillin (71.4%) and Chloramphenicol (71.4%) and least resistance was observed with Pefloxacin (50%), Tarivid (50%), Sparfloxacin (57%), Ciprofloxacin (50%) antibiotics. *Salmonella spp* showed the highest resistance to Septrin (100%), Augmentin (91%), Streptomycin (91%), Amoxacillin (83.3%), Chloramphenicol (83.3%), Tarivid (83.3%) and least resistance to Sparfloxacin (33.3%), Ciprofloxacin (41.6%) and Pefloxacin (41.6%) antibiotics. *Pseudomonas spp* showed (100%) resistance to Gentamycin, Augmentin, Amoxacillin, Ciprofloxacin, Sparfloxacin, Septrin and Streptomycin, though the resistance was observed to be slightly minimal to Pefloxacin (75%), as well as Tarivid and Chloramphenicol.

Discussion

The study was carried out to investigate the antibiotic susceptibility

pattern of Gram-negative bacteria isolated from infected wounds in two different health-care centers Gboko. The Gram negative bacteria isolated in the infected wounds in order of predominance were: *Escherichia coli, Salmonella spp* and *Pseudomonas spp.* The isolated Gram negative bacteriaare classified among medically important pathogens [18].

The presence of multidrug resistant pathogens in wounds of patients in Gboko town presents a major threat to human life in this part of the world since there is a dearth of proper information on MDR in Gboko LGA especially in the selected privately owned and operational hospitals studied. Our result of MDR test conducted on all the isolates from the two hospitals showed that *Escherichia coli* had the highest resistance to streptomycin (92.8%), Augmentin (92.8%), Septrin (78.5%), Gentamycin (78.5%), Amoxacillin (71.4%) and Chloramphenicol (71.4%) with low resistance observed with Pefloxacin (50%), Tarivid (50%), Sparfloxacin (57%), Ciprofloxacin (50%) antibiotics. *Salmonella spp* showed the highest resistance to Septrin (100%), Augmentin (91%), Streptomycin (91%), Amoxacillin (83.3%), Chloramphenicol (83.3%), Tarivid (83.3%) and least resistance to Sparfloxacin (33.3%), Ciprofloxacin (41.6%) and Pefloxacin (41.6%) antibiotics. *Pseudomonas spp* (100%) resisted Gentamycin, Augmentin, Amoxacillin, Ciprofloxacin, Sparfloxacin, Septrin and Streptomycin, though the resistance was observed to be slightly minimal to Pefloxacin (75%), as well as Tarivid and Chloramphenicol.

Previous studies of Kallen et al. [19] conducted on MDR among gram-negativebacteriaresponsible for healthcare related infections in Atlanta, Georgia (USA) established that 10% of *P. aeruginosa*, and 15% *K. pneumoniae* resisted three antimicrobial classes. A greater fraction, 60% of *Acinetobacter baumannii* isolates resisted at least 3 antimicrobial classes. Though less common, isolates with four class resistance were also observed in substantialamounts and through regions.

Pirvanescu et al. [20] reported in their studies that a rate of 27.6 bacterial strains isolated from samplesexhibited multi-drug resistance. The bacteria which included *Staphylococcus aureus*; both MRSA strains and ESBL Gram negative bacteria studied indicated high resistance to, quinolones, aminoglycosides, third generation cephalosporins and low resistance to fourth generation cephalosporins. Neither Vanomycin resistant *Staphylococcus aureus* strains nor vancomycin-intermediate strains were isolated from the samples.

Research reports of Paramythiotou and Routsi [21] which spanned from October 2014 to May 2015, isolated and identified 40 carbapenem resistant blood stream infections detected in patients that were on admission to Tata Medical Center, Kolkata, India. The isolates were *A. baumannii, E. coli, P. aeruginosa* and *K. pneumoniae*. These studies have corroborated our research findings and further confirm the health challenge posed by MDR Gram negative bacteria from wounds which calls for continuous and sustained surveillance to contain the upsurge. Resistance of bacteria pathogens to antimicrobial is an emergent global problem. Conversely, the amplified proportion of MDR realized in this study was considered as disturbing since only a few treatment options remain for wound infections by the isolated Gram negative bacteria.

The extensive usages of antibiotics, duration over which the drugs have been available for market have led to foremost complications of the advent of resistant bacteria [22]. Abuse of antimicrobial drugs, over dose, wrong drugs prescription with inappropriate susceptibility test, self-medication and long period of hospitalization was suggested as factors that could enhance the problem of MDR in unindustrialized nations, which Nigeria is inclusive [23].

Conclusion

There is an alarming increase of wound infection caused by antibiotic resistance bacteria. Lack of uniform antibiotic policy and indiscriminate use of antibiotic may have led to emergence of resistance bacterial strains. Particularly, *Pseudomonas spp* resistance to third generation antibiotics isa real threat to control hospital acquired infections. *Escherichia coli* is the most frequent pathogen implicated in this study with highest resistance rates compared to other bacterial isolates from infected wound followed by *Salmonella spp*.

Sustained periodical bacteriological monitoring and surveillance of pathogens isolated from patients in hospitals is essential to induce the attention of clinicians and infection control agencies and health policy makers to emerging antibiotic susceptibility pattern and how regularly specific pathogens are isolated. We suggest appropriate antimicrobial resistance surveillances and structured prevention approaches to curtail further spread of the pathogens in the study area. This will be of immense benefit to the patient and important to heath workers in administration of appropriate chemotherapy in the affected patients.

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