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Research Article

ETIOLOGY AND ANTIBIOGRAM OF BACTERIA OF PUBLIC HEALTH IMPORTANCE FROM WOUND INFECTIONS

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Abstract

Etiology and antibiogram of bacteria of public health importance from wound infection was investigated. A total of one hundred (100) wound samples were collected from different patients with wound infections in some hospitals in Owerri, Imo State, Nigeria using sterile swab sticks. Standard microbiological methods were adopted in the isolation and determination of the antibiogram of the bacterial isolates. A total of one hundred and thirty-eight (138) bacterial isolates comprising *Staphylococcus aureus* 39(28.3%), *Escherichia coli* 26(18.8%), *Pseudomonas aeruginosa* 22(15.9%), *Streptococcus mutans* 16(11.6%), *Proteus vulgaris* 17(12.3%) and *Proteus mirabilis* 18(13.0%) were isolated. Antibiogram of the bacterial isolates revealed zones of inhibition ranging from 12mm to 28mm. Some of the bacterial isolates were resistant to streptomycin, amoxicillin and gentamycin antibiotics. The percentage resistance of Gram positive organisms was 30%, and the percentage susceptibility was 70%. For Gram negative organisms, the percentage resistance of the organisms was 20%, while the percentage susceptibility was 80%.Proper diagnosis of wound infections is necessary before treatment in order to curb the high rate of antibiotic resistant organisms.

Keywords: Etiology, Antibiogram, Wound, Infection, Bacterial.

INTRODUCTION

Loss of skin integrity (trauma) caused by mechanical, biological or chemical agent provides a suitable environment for infectious agent entrance, colonization and consequent acute/chronic infection. Wound is a type of injury which happens relatively quickly in which skin is torn, cut or punctured. Wound provides a moist, warm, nutritive environment conducive to microbial colonization, proliferation, and infection (Mohammed et al., 2017).

Wound infections have been a problem in the field of medicine for a long time, and the problem complicated more recently because of increased antimicrobial resistance. Wound infection is also a problem for public, researchers, clinicians and drug companies looking for effective drugs (Weledji, 2012). Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality.

Wound infections can be caused by different groups of microorganisms like bacteria, fungi and protozoa. However, different microorganisms can exist in polymicrobial communities especially in the margins of wounds and in chronic wounds (Percevil and Bowler, 2004). The infecting microorganism may belong to aerobic as well as anaerobic group (Bowler, 2008). Wound contamination is characterized as the arrangement of discharge in an injury, and in addition other general or neighborhood components of sepsis including pyrexia, torment and in terms (Mordi and Momoh, 2009).

Wound diseases represent 70-80% death rate (Goellnsha et al., 2013). Wound may be countered in clinical practice either postoperatively, taking after injury, or could principally be of infective birthplace (Mohammed et al., 2017). Despite their starting point, all injuries may debase by microorganisms or outside bodies or both. Most commonly isolated

aerobic microorganisms include *Staphylococcus aureus*, Coagulase-negative *staphylococci* (CoNS), *Enterococci, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter* species*, Proteus mirabilis, Candida albicans* and *Acinetobacter* (Rajendra-Gautam et al.*,* 2013).

For effective wound management and treatment, understanding of the antibiotic susceptibility pattern of the infecting microorganisms is necessary in order to curb the increase in drug resistant organisms in wound sepsis. Hence, this study was carried out to determine the etiology and antibiogram of bacteria of public health importance from wound infections.

MATERIALS AND METHODS

Collection of Antibiotics Samples

Commercially available antibiotic discs were purchased from a pharmacy shop in Owerri, Imo State.

Collection of Wound Samples

A total of hundred (100) wound samples were collected from different patients with wound infection in some hospitals in Owerri, Imo State, Nigeria using sterile swab sticks. The swab sticks were labeled accordingly and were taken to the laboratory for isolation of bacteria associated with the wound samples.

Sterilization of Glass wares and Media

All the glasswares used in this study were sterilized using laboratory hot air oven at temperature of 160 ^{O}C for 1 hours and media (nutrient agar, eosin methylene blue agar, mannitol salt agar and blood agar) used in this study were prepared according to manufacturer's instruction and were sterilized using the autoclave at a temperature of 121^{0} C at 15 psi for 15 minutes. After the sterilization, the media were brought out together with the glassware and kept on a clean laboratory bench. The media were poured into the Petri dishes when cooled to 45 ^{O}C and allowed to solidify (Cheesbrough, 2010).

Isolation of bacteria from the wound infection samples

The method described by Ede et al. (2017) was adopted in the isolation of bacteria associated with wound infections in the wound samples used in this study. Each of swab sticks containing the wound samples was streaked onto nutrient agar, blood agar, eosin methylene blue agar and MacConkey agar. The plates were incubated inverted for 24 hours at a temperature of 37^{0} C.

Colonial Morphology Identification

The method described by Cheesbrough (2010) was adopted in the colonial morphology identification**.** Presumptive identification of the colonies was done by observing their individual shape, colour, elevation, edge, surface, consistency and appearance on the media used for isolation. Colonies with characteristic metallic sheen on EMB agar and lactose fermenters on MacConkey agar were noted. The colonies were preserved in sterile agar slants in test tubes.

Purification and Preservation of Isolates

After the various Colony counts, bacterial isolates were pick with a wire loop based on their cultural and morphological characteristics. The picked colonies were sub-cultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24hrs at 37^{0}C. After incubation pure cultures were stored in McCartney Bottle in a refrigerator (Cheesbrough, 2010; Ochei and Kolhatkar, 2010).

Typical colonies stored on nutrient agar slants at 4ºC were Gram stained and confirmed (Speck, 1976). Cultural characteristics and biochemical tests: motility, Oxidase, Catalase, Coagulase, sugar production test, citrate utilization test were carried out to identify and characterize the organisms Cheesbrough (2010).

Antibiogram of the Isolated Bacteria

Antibiotic susceptibility profiles of the bacterial isolates were evaluated using disk diffusion assay. The antibiotic discs containing the antibiotics (for Gram positive and Gram negative organisms respectively) were used. The discs were

aseptically placed on the surface of Mueller-Hinton agar (MHA) plates that had already been seeded with 0.5 McFarland standard of the test isolates and were incubated at 37°C for 18-24hrs. After incubation, diameters of zone of inhibitions were observed and measured in millimeters accordingly. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative table (CLSI, 2010).

RESULTS AND DISCUSSION

Table 1: Morphological and biochemical characteristics of the bacterial isolates from wound infections

Morphological Characteristics	Gram reaction	Oxidase test	Indole test	Spore test	Catalase test	Citrate test	Coaguase test	Motility test	S FT				Possible bacteria
									S	B	G	H_2S	
Milkish, raised, non- mucoid	Gram positive cocci	-	-	-	+	-	+	-	No Reaction		-	-	*Staphylococcus aureus* colonies
Pinkish, convex,	Gram negative rods	-	-	-	+	+	-	+	Y	Y	+	-	*Escherichia coli* non-mucoid colonies
Bluish-green, flat	Gram negative rods	+	-	-	+	-	-	+	R	R	-	-	*Pseudomonas aeruginosa* non-mucoid colonies in diploids
Milkish, raised, non- mucoid	Gram positive cocci	-	-	-	-	-	-	-	No Reaction		-	-	*Streptococcus mutans* colonies
Pale, flat, non-mucoid	Gram negative rods	-	-	-	-	+	-	+	R	Y	+	+	*Proteus mirabilis* elongated colonies in short chains
Pale, flat, non-mucoid	Gram negative rods	-	+	-	-	+	-	+	R	Y	+	+	*Proteus vulgaris* elongated colonies in short chains

KEY: -= Negative += Positive S= color of slope B= color of butt G= Gas production H2S= Hydrogen sulphide production (blackening) R= Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

Table 2: Occurrence of bacterial isolates from the wound infection

Bacterial isolates	Occurrence	Percentage
S. aureus	39	28.3
E. coli	26	18.8
P. aeruginosa	22	15.9
S. mutans	16	11.6
P. vulgaris	17	12.3
P. mirabilis	18	13.0

Table 3: Zones of Inhibition of the antibiotics against the Gram positive bacteria from wound infections

Organisms	Zones of inhibition (mm)/Antibiotics used									
	NB	CH	CPX	E	LEV	CN	RD	AMX	S	APL
	(Gram positive)									
Staphylococcus aureus	-	17	20	18	26	18	-	-	22	26
Streptococcus mutans	14	20	21	-	25	20	24	-	-	20

Key: NB = Norfloxacin (10mcg) CH = Chloramphenicol (30mcg)
CPX = Ciproflax (10mcg) E = Erythromycin (30mcg)
LEV = Levofloxacin (20mcg) CN = Gentamycin (10mcg)
RD = Rifampicin (20mcg) AMX = Ampiclox (20mcg)
S = Streptomycin (30mcg) APL = Amoxil (20mcg)
CLSI standard: R= Resistant (0-12mm)
I= Intermediate (12-16mm) S= Susceptible (16mm and above)

Table 4: Zones of Inhibition of the antibiotics against the Gram negative bacteria from wound infections

Organisms	Zones of inhibition (mm)/Antibiotics used									
	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
	(Gram negative)									
Pseudomonas aeruginosa	28	24	26	16	12	22	24	28	24	16
Escherichia coli	18	18	26	18	-	-	18	16	22	20
Proteus vulgaris	24	21	20	22	22	24	24	28	24	26
Proteus mirabilis	28	26	26	22	22	24	24	28	24	26

Key: OFX = Tarivid (10mcg) PEF = Riflacine (10mcg)
AU = Augmentin (30mcg)
CN = Gentamycin (10mcg) S = Streptomycin (30mcg)
CEP = Ceporex (10mcg) NA = Nalidixic acid (30mcg)
SXT = Septrin (30mcg) PN = Ampicillin (30mcg)
CLSI standard: R= Resistant (0-12mm)
I= Intermediate (12-16mm) S= Susceptible (16mm and above).

Bacteria are the major contaminants of wound infections. This study evaluated the etiology and antibiogram of bacteria of public health importance from wound infection. The results of this study are shown in Table 1 to 4. Table 1 shows the result for cultural morphology and biochemical characteristics of the bacterial isolates from the wound infections. A total of one hundred and thirty-eight (138) bacterial isolates comprising *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Proteus vulgaris* and *Proteus mirabilis* were isolated.

Rajendra-Gautam et al. (2013) reported the isolation of wound infections of *Staphylococcus aureus*, Coagulase-negative *staphylococci* (CoNS), *Enterococci, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter* species*, Proteus mirabilis, Candida albicans* and *Acinetobacter* species. Similarly, Atiyeh et al. (2007) reported the isolation of *Escherichia coli*, *Pseudomonas* species, *Proteus* species and *S. aureus* from wound swabs collected from surgical unit a hospital. The results of this study are similar to their report.

S. aureus is a Gram-positive bacterium which is a major pathogen implicated in skin infections such as impetigo, furuncles, boils, sties, pustules, burns, and wounds. *Escherichia coli* has been involved in wound infections, urinary tract infections and other infections in humans. *Pseudomonas aeruginosa* is implicated in skin infections and other infections (Ochei and Kolhatkar, 2010).

The occurrence of the bacterial isolates from the wound infections are shown in Table 2. *Staphylococcus aureus* 39(28.3%) was the most prevalent bacterium followed by *Escherichia coli* 26(18.8%), and *Pseudomonas aeruginosa* 22(15.9%), *Streptococcus mutans* 16(11.6%), *Proteus vulgaris* 17(12.3%) and *Proteus mirabilis* 18(13.0%). The result of the antibiogram of the Gram positive bacteria from the wound infections is shown in Table 3. The zones of inhibition ranged from 14mm to 26mm with levofloxacin being the most effective antibiotic followed by amoxcil. Both *S. aureus* and *S. mutans* were resistant to ampiclox, though the organisms were separately resistant to two other antibiotics. The percentage resistance of the organisms was 30%, and the percentage susceptibility was 70%.

The result of the antibiogram of the Gram negative bacteria from the wound infections is shown in Table 4. The zones of inhibition recorded ranged from 12mm to 28mm with Tarivid being the most effective antibiotic followed by septrin and nalidixic acid. *Pseudomonas aeruginosa* was the most susceptible bacterium followed by *Proteus mirabilis,* while *E. coli* was the most resistant organism. The percentage resistance of the organisms was 20%, while the percentage susceptibility was 80%. Drug resistance recorded in this study is of public health concern as antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality.

The presence of multidrug resistant pathogens in wounds of patients presents a major threat to human life in this part of the world. Previous studies of Alexandra et al. (2010) conducted on multi-drug resistance (MDR) among gram-negative bacteria responsible 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. Pirvanescu et al. (2014) reported in their studies that a rate of 27.6 bacterial strains isolated from wound samples exhibited multi-drug resistance. The bacteria which included *Staphylococcus aureus* 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.

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 (Buteera and Byimana, 2009). 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 (Nkang et al*.,* 2009).

To ensure fast healing of wound infection as well as drug abuse and misuse in the society, people are advised to go for laboratory diagnosis that will determine the causative organism of infection so as to determine the antibiotics that could be effective against the causative organisms.

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