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Antibiotic Sensitivity Pattern of Bacteria Associated With Wound Infections in Kaduna Metropolis

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Abstract

Chronic wounds infections could result to structural damage and establishment of a chronic biofilm which stimulates host immune response that cause further damage generating a vicious cycle. Bacteria pathogens associated with wound infections were characterised and their resistance profile to the most common conventional antibiotics generated. Fifty (50) wound samples were collected from 50 patients and were screened on blood agar and MacConkey agar, while Mueller Hilton agar was used for the determination of antibiotics susceptibility test using kirby Bauer disc method. The predominant bacteria isolated were *Staphylococcus aureus* (50%) followed by *Escherichia coli* (36%), *Pseudomonas aeruginosa* (30%), *Klebsiella pneumonia* (16%), *Streptococcus pyogenes* (8%), *Proteus mirabilis* (4%) and least by *Enterococcus feacalis* (2%). Gram negative bacteria presented (58.9%) compared to their Gram positive counterpart which had 41.1% prevalence. Gram-negative bacteria were resistant to ampicillin. Gram- negative bacteria showed quite high resistant to the majority of antibiotics used in this research, while some were active against these bacteria. The antibiotics used on Gram negative bacteria in this research were; Ampicillin, Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxacillin, Augmentin, Gentamycin, Pefloxacin, Tarivid and Streptomycin. While for Gram positive are; Ampicilin, Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Recephin, Amoxacillin, Ciprofloxacin, Streptomycin, Septrin and Erythromycin. The knowledge of agents of wound infections and the antibiotic sensitivity test as was seen from this research could be viable tool in the selection of antibiotic therapy and infection control measures in public health care and policies regarding antibiotic utilization.

Key: Wound, Immunosuppressants, Exogenous infection, Endogenous infection, Nosocomial infection, Antibiotics

INTRODUCTION

The primary function of the intact skin is to control microbial population that live on the skin surface and to prevent underlying tissues from becoming colonized and invaded by potential pathogens. Wound results due to the disruption of the skin which is usually accidentally. Wound care constitutes an important part of routine care given by health professionals to the community population (Meaume, 2012).

The breaking of the host protective layer- the skin, and thus disrupting the protective functions of the layer, will induce many cell types into the wound to initiate host response (Collier, 2003). An effective management of wounds especially chronic wounds in the health care setting can have an impact in the population health, reducing morbidity and improving function and quality of life (Bessa, 2013).

However, wound infections can be exogenous or endogenous infection. In exogenous infection, the causal organism comes from elsewhere which could be as a result of contamination by animate or inanimate objects such as during surgery or wound dressing. Endogenous infections are caused by opportunistic pathogens that have been in existence on the patient's body (Collier, 2003). For example, in a case of colon cancer infection, it could be as a result of opportunistic flora of the intestinal origin (Singleton, 2009).

In spite of technological advances that have been made in surgery and wound management, wound infections have been regarded as the common nosocomial infection (Dionigi, 2001). It is an important cause of illness resulting to prolonged hospital stay, increased trauma care, treatment cost and thus general wound management becomes demanding (Bowler, 2001). Also wound infection delays healing process and may cause wound breakdown, (Alexander, 2000). However, health is not a germ-free state but rather a delicate balance between host resistance and numerous species of bacterial which are present at all times.

The knowledge of the causality agents of wound infections has therefore proved helpful in the selection empiric antimicrobial therapy and on infection control measures in health institutes (Shittu, 2005). The control of wound infections became challenging due to widespread of bacterial resistance to antibiotics and to a greater incidence of infection caused by Methicillin-resistance *Stapylococcus aureus* (MRSA), nosocomial infections, polymicrobic flora and other organisms (Collier, 2003). The world wide escalation in both community and hospital acquired antimicrobial resistance bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance; more appreciate antimicrobial prescription, prudent infection control and new treatment alternatives (Alawode, 2001).

The introduction of antibiotics has reduced morbidity rate which were on the high side due to life threatening diseases especially wound infections (Collier, 2003). Enlightenment on the use and management of antibiotics cannot be over emphasized as it will continue to be the way out of many infections which occur frequently in human. Increase in the misuse and management of antibiotics which are now leading to drug resistance which is creating a lot of concern in medical practice (Collier, 2003).

Determination of bacteria sensitivity pattern to antibiotics is important in providing a guide for antibiotic selection (Elamanya, 2014). Used appropriately systemic, antibiotics or combination of antibiotics do have an important potentially lifesaving role in the management of wound infection. Administrating antibiotic or combination of antibiotic maybe necessary in which intravenous, intramuscular or orally are usually administered or given to patients with infected wounds (Imanikandan, 2013).

There are factors that increase the risk of wound infection which include patient Characteristics such as; age, obesity, malnutrition, endocrine and metabolic disorders, smoking, hypoxia, anaemia, malignancies and immune suppressants (Giacometti, 2000). Other factors are the state of the wound which includes nonviable tissue in the wound, foreign bodies and formation of haematomas, long surgical procedures, and contamination during operation, poor surgical techniques, hypothermia and prolonged pre-operative stay at the hospital (Giacometti, 2000).

Wound infections can be prevented by restoring blood circulation as soon as possible, relieving pain, maintaining normal body temperature, performing surgical toilet and debridement of the wound as soon as possible, administration of antibiotic prophylaxis for deep wound and high risk infections (Giacometti, 2000). High risk wounds include contaminated wounds, penetrating wounds, abdominal trauma, compound fractures, wounds with devitalized tissue; high risk anatomical sites such as hands and feet. Establishment of the causative microorganism is important and treatment should be initiated based on the bacterial sensitivity patterns (Imanikandan, 2013).

MATERIALS AND METHODS

Study Area

A prevalence rate of 25% in a previous research was use to arrived at a total sampling size fifty (50) wound swab and were collected from hospitals within Kaduna metropolis.

Collection of Samples and Analysis

Fifty (50) wound swabs samples were collected from hospitals within Kaduna metropolis which include; Dantsoho Memorial Hospital and 44 barrack hospitals. A clean sterile swab sticks were then used to swab the affected area, labelled and transported to the Department of microbiology laboratory, Kaduna state university, cultured and smears were made on clean grease free glasses slides for Gram staining.

Media Preparation

All media were prepared according to the manufacturer's specification. All the media prepared were sterilized by autoclaves at 121^{0}C for 15 minutes. MacConkey agar, blood agar and Mueller Hinton were prepared according to manufacturer instruction. After which were allowed to cool for about 45^{0}C and then were dispensed aseptically into sterile Petri-dishes and were allowed to solidify (Imanikandan, 2013).

Isolation of Microorganisms from Wound Infections

Each sample collected was inoculated on both differential and enriched media (MacConkey agar and blood agar respectively) the inoculums on the plates were streaked out for discrete colonies with sterile wire loop. The culture plates were incubated aerobically at 37^{0}C for 24 hours before colonial morphologies were identify and interpreted using microbiology atlas.

Characterization and Identification of Microorganisms

Preliminary identification of bacteria were based on colony characteristics of the organisms i.e. haemolysis on blood agar and changes in appearance in differential media (Imanikandan, 2013). Biochemical test were performed on colonies from primary cultures for final identification of isolates.

Antibiotic Susceptibility Test

A multi disc of blotting paper containing a measured quantity of the microbial disc was placed on the surface of a solid medium that has been inoculated on the surface with the test organism and incubated at $37^0$0 for 24 hours. The antibiotic diffuses from the disc into the medium and the growth of the test organism was inhibited at a distance from the disc that is related the sensitivity of the organism (Colle, 2002). The diameter (mm) is of the zones of inhibition were measured using a meter rule and interpreted as sensitive (16 to 20mm and above), intermediate (10 to15mm) and resistance (0 to 9mm). Drug (antibiotics) tested against the isolates (Cheesbrough, 2006; Kirby, 2014).

Results

Table 1 shows the cultural characteristics and biochemical characteristics of bacteria isolated from wounds and their Gram reaction. *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumoniea* were lactose fermenters unlike *Proteus mirabilis* that was not lactose fermenting on MacConkey agar. *Streptococcus pyogenes* did not grow on MacConkey agar. *Staphylococcus aureus, Enterococcus faecalis,* and *Streptococcus pyogenes* were Gram positive bacteria while *Pseudomonas aeruginosa, Escherichia coli, Klebsiella. Pneumoniea* and *Proteus mirabilis* were Gram negative bacteria.

The biochemical characterisation of the bacteria isolates; the biochemical characteristics were used to identify the following isolates from wound infections, which include; *Staphylococcus aureus, Escherichia coli, Proteus* mirabilis, *Klebsiella* pneumoniea, *Streptococcus pyogene*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*.

Table 2 shows the occurrence and frequencies of bacteria isolated from wound infections as follows. *Stapylococcus aureus* 25 (50%), *Escherichia. coli* 18 (36%), *Pseudomonas aeruginosa* 15 (30%), *Klebsiella Pneumoniea* 8 (16%) *Streptococcus pyogenes* 4 (8%), *Proteus mirabilis* 4 (8) and *Enterococcus faecalis* 1(2%).

Table 3 shows the antibiogram for Gram negative bacteria isolated from different wounds infection. All the Gram negative bacteria were resistance to Ampicillin, some were resistance to Amoxacillin while Chloramphenicol, Septrin and Ciprofloxacin are susceptible to the Gram negative bacteria.

Table 4 present the antibiogram for Gram positive bacterial isolated from different wounds infection. All the Gram positive bacteria were resistance to Ampicillin, some were resistance to Amoxacillin and Streptomycin while Zinnaccef and Ciprofloxacin are susceptible to the Gram positive bacteria

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Table 1:Cell Morphology and Biochemical Characteristic of Bacteria Isolated from Wound Infections

Cell morphology	Gram reaction	Growth on B.A	Growth on MCA	Motility	Catalase	Coagulase	Oxidase	Citrate	Urease	Indole	TSI	Sugar fermentation G	S	L	Probable organisms
Cocci	+	Cream, beta haemolytic	Raised colonies	-	+	+	-	+	+	-	A/A	A	A	A	*Staphylococcus aereus*
Rod	-	Smooth Greenish colonies	Pink smooth colonies	+	+	-	+	+	-	-	K/K	-	-	-	*Pseudomonas aeruginosa*
Rod	-	Cream, muciod	Smooth pink	+	+	-	-	+	-	+	A/A	A/G	A/G	A/G	*Escherichia coli*
Rod	-	Large white	Pink,muciod	-	+	-	-	+	+	+	A/K	A/G	A	A	*Klebsiella pneumoniea*
Cocci	+	Greenish, alpha haemolytic	No growth	-	-	-	-	+	-	-	A/A	A	A/G	A/G	*Strept*. pyogenes
Rod	-	Blue grey	Blue grey	+	+	-	-	-	+	-	A/A	A	-	-	*Proteus mirabilis*
Cocci	+	Cream, beta haemolytic	Smooth pink	-	-	-	+	+	+	+	A/A	A/G	A	A	*Enterococcus fsaecalis*

KEY: BA; Blood Agar, MCA; MacConkey Agar, TSI; Triple salt Iron, A; Acid, K; Alkaline, +; Positive, -; Negative

Table 2: Frequency of Occurrence of Bacteria Isolated from Wound Infections

Microorganisms	No. of Occurrence	No. Positive	Frequency (%)
Staphylococcus aureus	25	25	50
Pseudomonas aeruginosa	15	15	30
Escherichia coli	18	18	36
Klebsiella pneumoniea	8	8	16
Streptococcus pyogenes	4	4	8
Proteus mirabilis	2	2	4
Enterococcus faecalis	1	1	2

Table 3: Antibiotic Sensitivity Pattern of Gram Negative Bacteria Associated with Wound Infections

Antibiotics		*P. mirabilis*			*P. aeruginosa*			*K. pneumoniea*			E. coli			Total	Resistant %	Intermediate %	Sensitive %
Antibiotics	Strength	R	I	S	R	I	S	R	I	S	R	I	S				
Septrin	30µg	1(R)	4(I)	6(S)	10(R)	1(I)	4(S)	6(R)	-	2(S)	10(R)	2(I)	6(S)	43	62.8	16.2	41.9
Chloramphenicol	30µg	-	-	2(S)	1(R)	4(I)	10(S)	3(R)	2(I)	3(S)	5(R)	3(I)	10(S)	43	20.9	20.9	58.1
Sparfloxacin	10µg	1(R)	1(I)	-	10(R)	1(I)	4(S)	7(R)	1(I)	-	2(R)	3(I)	13(S)	43	46.5	14.0	39.7
Ciprofloxacin	10µg	1(R)	-	1(S)	9(R)	1(I)	5(S)	5(R)	-	3(S)	6(R)	3(I)	9(S)	43	37.2	9.3	41.9
Amoxicillin	30µg	1(R)	1(I)	-	13(R)	1(I)	1(S)	6(R)	1(I)	1(S)	10(R)	3(I)	5(S)	43	69.8	14.0	16.3
Ampicillin	30µg	2(R)	-	-	15(R)	-	-	8(R)	-	-	18(R)	-	-	43	100	-	-
Augmentin	30µg	-	2(I)	-	8(R)	1(I)	6(S)	1(R)	4(I)	3(S)	9(R)	5(I)	4(S)	43	41.9	27.9	30.2
Gentamycin	10µg	1(R)	-	1	9(R)	4(I)	2(S)	5(R)	1(I)	2(S)	8(R)	4(I)	5(S)	43	41.9	20.9	23.3
Pefloxacin	30µg	-	-	2(S)	1(R)	10(I	4(S)	4(R)	2(I)	2(S)	5(R)	6(I)	7(S)	43	23.3	41.9	34.9
Tarivid	10µg	-	1(I)	1(S)	5(R)	6(I)	4(S)	6(R)	2(I)	-	5(R)	5(I)	8(S)	43	37.2	32.9	30.6
Streptomycin	30µg	1(R)	-	1(S)	10(R)	2(I)	3(S)	1(R)	3(I)	4(S)	4(R)	10(I)	4(S)	43	37.2	34.9	27.9

KEY: R= Resistance, S= Susceptible, I= Intermediate, - = No result Total= No of Bacteria tested.

Table 4: Antibiotic Sensitivity Pattern of Gram Positive Bacteria Associated with Wound Infections

Antibiotics		*Streptococcos pyogenes*			*Staphylococcus aureus*			*Enterococcus* Feacalis			Total	Resistant %	Intermediate %	Sensitive %
Antibiotics	Strength	R	I	S	R	I	S	R	I	S				
Pefloxacin	10µg	3(R)	-	1(S)	5(R)	10(I)	10(S)	-	-	1(S)	30	26.7	33.3	40.0
Gentamycin	10µg	2(R)	-	2(S)	5(R)	7(I)	13(S)	1(R)	-	-	30	26.7	23.3	50.0
Ampiclox	30µg	4(R)	-	-	3(R)	10(I)	12(S)	1(R)	-	-	30	26.7	30.3	40.0
Zinnacef	20µg	-	1(I)	3(S)	2(R)	5(I)	18(S)	-	-	1(S)	30	6.7	20.0	73.3
Amoxicillin	30µg	4(R)	-	-	20(R)	2(I)	3(S)	-	1(I)	-	30	86.6	10.0	10.0
Recephin	25µg	2(R)	2(I)	-	10(R)	-	10(S)	5(R)	-	1(S)	30	40	6.7	36.7
Ampicillin	30µg	4(R)	-	-	25(R)	-	-	1(R)	-	-	30	100	-	-
Ciprofloxacin	10µg	1(R)	1(I)	2(S)	2(R)	4(I)	19(S)	-	1(I)	-	30	10	20.0	70.0
Streptomycin	30µg	4(R)	-	-	16(R)	5(I)	4(S)	-	1(I)	-	30	66.7	20.0	13.3
Septrin	30µg	2(R)	1(I)	1(S)	15(R)	4(I)	5(S)	1(R)	-	-	30	60	16.7	20.0
Erythromycin	10µg	2(R)	-	2(S)	6(R)	9(I)	10(S)	1(R)	-	-	30	30	30.7	40.0

KEY: R= Resistance, S= Susceptible, I= Intermediate, - = No result and Total= No of Bacteria tested.

Discussion

This study revealed that the bacteria isolated from wound infections were variable.

The 50% frequency of occurrence of *Staphylococcus aureus* could be majorly the cause of wound infections due to the predominant frequency. In addition, *Staphylococcus aureus* has been reported to be the normal flora of the skin (Imanikandan, 2013). and hence the high frequency obtained in the study. Some of the bacteria isolated from wound infections from this research *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, and Escherichia coli* are similar to the work of Elemanya, 2004 who also reported the occurrence of these bacteria in wound infection. Only few of the wounds samples were polymicrobic in nature and in most cases associated with *Staphylococcus aureus* as the predominant bacteria. The second predominant bacteria, *Escherichia coli* in wound infection obtained in this research could be due to the ability of the bacteria to survive under variety of conditions and are member of the normal flora of the gastrointestinal tract as reported by Imanikandan, (2013).

The occurrence of other bacteria such as *Streptococcus pyogenes, Klebsiella pneumoniea, Enterococcus faecalis, Pseudomonas aeruginosa* and *Proteus mirabilis* from this study could be due to contamination of the wound with endogenous flora. Similarly, other researchers have reported the infection of open wounds due to environmental contaminants (Giacometti, 2000; Imanikandan, 2013; Gurusamy *et al.*, 2013b). Although the predominant organism, *Staphylococcus aureus* which is a Gram positive bacteria, but never the less Gram negative bacteria were dominant from this research (58.9%) compare to their Gram positive counterpart which has 41.1% prevalence. Other researchers have also reported that Gram negative bacteria could be responsible for wound infections (Imanikandan 2013, Gurusamy *et al.*, 2013b).

High rate of drug resistance to antibiotics were found in most of the bacteria isolated.

All Gram negative and Gram positive bacteria are resistance to Ampicillin, this could be due to prolonged, drug abuse of antibiotics and their oral route of administration which affect their rate of absorption into blood stream led to the emergence of antibiotics resistance in Bacteria as reported by (Imanikandan, 2013 & Giacometti, 2000). In addition, Self-medication, expired antibiotics, counterfeit drugs and inadequate hospital control measure promote the development of resistance in clinical isolates from wound infection.

Most Gram positive bacteria are susceptible to Zinnacef and ciprofloxacin while Gram negative bacteria are susceptible to Chloramphenicol, Septrin and Ciprofloxacin. Ciprofloxacin is a third generation Cephalosporin that is relatively rare in the hospitals and have being less readily available for patients, hence more effective to patients using it. This is similar to the finding of Imanikandan (2013), Giacometti (2000) which revealed that emergence of antibiotics resistance in bacteria can be due to prolonged, unskilled practitioners, and inappropriate use of antibiotics for a long period of time.

Five out of fifty (50) wound swab samples had no bacterial growth. This could be due to normal healing process where the bacteria have been susceptible by body defence mechanism, antimicrobial activity or adequate nursing for the wound e.g. the use of antiseptics for cleaning of wounds or other conditions that could not support the growth of bacteria.

Conclusion

This research has shown the predominant bacteria associated with wound infections to be *Staphylococcus aureus* (50%),followed by *Escherichia coli* (36%), *Pseudomonas aeruginosa* (30%), *Klebsiella pneumonia* (16%), *Streptococcus pyogenes* (8%), *Proteus mirabilis* (4%) and least *Enterococcus feacalis* (2%). The most effective antibiotics were Chloramphenecol, Septrin, Ciprofloxacin and Zinnacef. However none of the isolated bacteria was susceptible to Ampicillin.

The knowledge of agents of wound infections and the antibiotic sensitivity test carried out from this research could be a viable tool in the selection of antibiotic therapy and infection control measures in public health care and policies regarding antibiotic utilization which may probably help to limit the increasing rates of drug resistance in pathogens.

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