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Isolation and Antibiotic Profile of Bacteria Associated with Wound Sepsis of Patients at Federal Medical Centre, Umuahia, Abia State

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Abstract

This work was conducted to isolate and identify bacterial species associated with wound sepsis and their sensitivity patterns to antibiotics. Wound swabs collected from twenty patients were processed to isolate bacterial species using standard techniques. Their sensitivity to antibiotics was also determined. Male and female patients ≥ 47 years and make patients aged between 35-40 years had 25% prevalence rate. 50% prevalence rate was recorded among male patients within the age range of 41-46 years. *Staphylococcus aureus* was the most prevalent bacterial agent (61.1%), followed by *Streptococcus* spp. (22.2%), while the least was *Pseudomonas* spp. (16.7%). 86.4% of *Staphylococcus aureus* was sensitive to Levofloxacin; and 59.1%was sensitive to Rifampicin. 86.4% of *Staphylococcus aureus* was resistant to Amoxil and Erythromycin respectively and 77.3% was resistant to Streptomycin. All the isolates of *Streptococcus* spp. were sensitive to Levofloxacin but were resistant to Ciproflox, Gentamycin, Amoxil, and Erythromycin (100%). *Pseudomonas spp*. showed 50% susceptibility to Streptomycin and 33.3% to Ciproflox. 83.3% of the isolates were resistant to Tarivid and Gentamycin while 50% were resistant to Ciproflox and Streptomycin. Treatment guidelines for use of antibiotics should be formulated based on the hospital formulary and the sensitivity patterns. This should be reviewed occasionally to ensure rational use of antibiotics.

Keywords: Isolation, Antibiotic Profile, Associated bacteria, Wound Sepsis.

1.0. Introduction

Wound infections have been regarded as the most common nosocomial infections and are associated with increased morbidity and mortality. Dionigi, Rovera, Dionigi, Imperator, Ferrari & Dionigi (2001); Iroha, Amadi, Orji & Esimone (2008); Akinjogunla, Adegoke, Mboto, Chukwudebelu & Udokang (2009).

Infection in a wound delays healing, causes wound breakdown, prolonged hospital stay, increased trauma care and treatment costs Bowler, Duerden & Armstrong (2001). Bacteriological studies have also shown that wound infections is universal and that the types of bacteria vary with geographical locations, bacteria resident on the skin, clothing at the site of wound, time between wound and examination. (Akinjogunla *et. al.,* 2009; Trilla, 1994). The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and due to an increasing incidence of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and polymicrobic flora. (Akinjogunla *et. al.,* 2009).

In developing countries, wound infections are recognized as a prominent route of bacterial infections. Many bacterial agents are known to cause wound infections. Yah, Enabulele & Eghafona (2004). Isolates that have been incriminated in cases of wound infections include: *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella aerogenes, Escherichia coli, Staphylococcus epidermidis, Streptococcus pyogenes,* and *Streptococcus faecalis. Candida albicans* and *Candida tropicalis* have also been implicated as etiological agents. Amelski & Barcynski (2002); Enweani, Esumeh, Akpe, Tatfeng & Isibor (2003); Kaplan, Smadi, Taani & El-Qudah (2000); Isibor, Oseni, Eyaufe, Osagie & Turay (2008).

Wound sepsis occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses. Characteristic local responses are a purulent discharge or painful spreading erythema indicative of cellulitis around a wound (Peel, 1992). Most acute and chronic wound infections involve mixed populations of both aerobic and anaerobic microorganisms.

Since wound colonization is most frequently polymicrobial (Bowler, (1999); Brook & Frazier (1997); Mousa, (1997); Summanen, Talan, Strong, Mcteague, Bennion & Finegold (1995), involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected. Although microorganisms are responsible for wound infection, widespread controversy still exists regarding the exact mechanisms by which they cause infection and also their significance in non-healing wounds that do not exhibit clinical signs of infection. One school of thought is that the density of microorganisms is the critical factor in determining whether a wound is likely to heal (Heggers, 1998; Mangram, Horan, Pearson, Silver & Jarvis (1999); Raahave, Friis-Moller, Bjerre-Jespen, Thiis-Knudsen & Rasmussen (1986), Robson, 1999). However, a second school of thought argues that the presence of specific pathogens is of primary importance in delayed healing (Danielsen, Balslev, Doring, Hoiby, Madsen & Westh (1998); Lavery, Harkles, Felder-Johnson & Mundine (1996); Madsen, Westh, Danielsen & Rosdahl (1996); Pallua, Fuchs, Hafemann, Volpel, Noah & Lutticken (1999); Schraibman, 1990; Sehgal & Arunkumar (1992), while yet others have reported microorganisms to be of minimal importance in delayed healing (Annoni, Rosina, Chiurazzi & Ceva (1989); Eriksson, Eklund & Kallings (1984); Gilchrist & Reed (1989); Hansson, Hoborn, Moller & Swanbeck (1995); Sapico, Witte, Canawati, Montgomerie & Bessman (1984); Trengove, Stacey, Mcgechie & Mata (1996). This study therefore is aimed at isolating, and identifying the bacteria species associated with wound infection as well as determining their antibiogram.

2.0. Materials and Methods

2.1 Sample Collection

A total of 20 samples (post-operative, abscess, bruises, burns) were collected from patients within the age range of 35 years and above at the Federal Medical Centre, Umuahia with the aid of a sterile swab stick with a normal saline and was later transferred to the microbiology laboratory section of the Daughters of Mary, Mother of Mercy Hospital, Ahiaeke, Abia State for analysis within one hour. A total of four wound swabs were collected from each patient,one of the wound swabs was used to make film and stained by Gram's stain. The second to fourth was cultured onto Blood agar, Nutrient agar and MacConkey agar respectively and incubated for 24 to 48 hours at 35-37^{o}C.

2.2 Isolation Methods

All specimen collected were immediately applied unto freshly prepared Nutrient agar, Blood agar, and MacConkey agar, streaked and incubated overnight at 37^{o}C for 24hrs. After incubation, the bacterial colonies were observed and discrete colonies were picked and purified by sub-culturing onto fresh Nutrient agar, MacConkey agar, and Blood agar using a streak plate technique. Isolated colonies that grew on the plates were then transferred onto Nutrient agar slants with a proper label. These agar slants were stored in the refrigerator at 4^{o}C and were used for further characterization.

2.3 Characterization and Identification of Bacterial Isolates

Characterization and identification of bacterial isolates was based on standard microbiological method including Gram staining, morphological and cultural characteristics on Nutrient agar media, catalase test, coagulase test, and indole production test, etc.

2.4 Antibiotic Sensitivity Test

The antimicrobial susceptibility testing was done on Mueller Hinton agar by the Kirby Bauer disc diffusion method and results were interpreted according to National Committee for Clinical Laboratory Standards guideline (NCCLS, 2004). A colony of the test organisms was picked and streaked on the agar respectively. A sterile forceps was used to pick the commercial antibiotic disc and placed on the medium. Zones of inhibition after 24hrs incubation at 35-37^{o}C were observed and measured using a caliper.
The antibiotics and their concentrations were as follows

Gram-positive antibiotic disc:

Ciproflox-10 µg,
Norfloxacin-10 µg,
Gentamycin-10 µg,
Amoxil-20 µg,
Streptomycin-30 µg,
Rifampicin20 µg,
Erythromycin-30 µg,
Chloramphenicol-30 µg,
Ampiclox-20 µg,
Levofloxacin-20 µg.
Septrin- 30 µg,

Gram-negative antibiotic disc:

Tarivid-10 µg,
Reflacine-10 µg,
Ciproflox-10 µg,
Augmentin-30 µg,
Gentamycin-10 µg,
Streptomycin-30 µg,
Ceporex-10 µg,
Nalidixic acid-30 µg,
Amplicin- 30 µg.

3.0. Results

A total of 20 wound samples were analyzed in this study. Bacterial isolates were obtained from 12 wound samples, while 8 samples had an insignificant growth. Biochemical tests carried out reveals the organism to be *Staphylococcus aureus, Streptococcus* spp., *Pseudomonas* spp. this is shown in Table 1. The frequency at which this isolates occurred by age and sex was depicted in Table 2. Majority of the isolates were from females within the age range of 35-40 and 41-45 (37.5 % respectively), while the least were isolated from males within the age range of 35-40 and ≥45 (25 % respectively). The most frequently isolated organisms were *Staphylococcus aureus* 22 (61.1 %), followed by *Streptococcus* spp. 8 (22.2 %), and *Pseudomonas* spp. 6 (16.7 %) in that order (Table 3).

Table 4 shows the occurrence and distribution of isolates in relation to the wound type. Out of the 36 isolates from the wound samples, 11 were isolated from abscesses, 9 from burns, while 8 were isolated from post-operative wounds and bruises respectively. *Staphylocoocus aureus* and *Streptococcus* spp. were isolated from all the wound sites while *Pseudomonas* was isolated from only burns and abscesss.

The antibiotic sensitivity and resistance patterns of the isolates was demonstrated on Table 5 and 6. 86.4% of *Staphylococcus aureus* was sensitive to Levofloxacin, and 59.1% was sensitive to Rifampicin. 86.4 % of *Staphylococcus aureus* was resistant to Amoxil and Erythromycin respectively and 77.3 % was resistant to Streptomycin. All the isolates of *Streptococcus* spp. were sensitive to Levofloxacin but were resistant to ciproflox, gentamycin, amoxil, and erythromycin (100 %). *Pseudomonas* spp. showed susceptibility to streptomycin at 50 % and 33.3 % to ciproflox. It was 83.3 % resistant to Tarivid, gentamycin, and 50 % resistant to ciproflox and streptomycin.

Table 1: Characterization and Identification of Isolates from Wound Sepsis

Sample Code	Colony Appearance	Shape	Gram Reaction	CA.	CI.	CO.	IN.	MR.	VP.	OX.	UR.	Probable Isolates
1a	Yellowish-white	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
1b	Creamy-white	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
1c	Colourless	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
2a	Yellowish-white	Cocci (scattered)	Positive	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
2b	Creamy-white	Cocci (scattered)	Positive	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
2c	Colourless	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
3a	Yellowish-white	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
3b	Colourless	Rod (scattered)	Negative	-	+	-	-	-	-	+	-	*Pseudomonas spp.*
3c	Greenish-blue	Rod (scattered)	Negative	-	+	-	-	-	-	+	-	*Pseudomonas spp.*
4a	Yellowish-white	Cocci (scattered)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
4b	Colourless	Cocci (chains)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
4c	Greenish-blue	Rod (chains)	Negative	-	+	-	-	-	-	+	-	*Pseudomonas spp.*
5a	Yellowish-white	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*

5b	Yellowish-white	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
5c	Colourless	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
6a	Creamy-white	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*
6b	Colourless	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*
6c	Colourless	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*
7a	Yellowish-white	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*
7b	Colourless	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*
7c	Greenish-blue	Rod (chains)	Negative	-	+	-	-	-	-	+	-	*Pseudomonas spp*
8a	Colourless	Cocci (scattered)	Positive	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
8b	Yellowish-white	Cocci (scattered)	Positive	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
8c	Colourless	Cocci (scattered)	Positive	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
9a	Yellowish	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
9b	Colourless	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
9c	Colourless	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
10a	Yellowish-white	Cocci (clusters)	Positive	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
10b	Colourless	Rod (scattered)	Negative	-	+	-	-	-	-	+	-	*Pseudomonas spp.*
10c	Greenish-blue	Rod (scattered)	Negative	-	+	-	-	-	-	+	-	*Pseudomonas spp.*
11a	Creamy-white	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*
11b	Yellowish-white	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*
11c	Colourless	Cocci (chains)	Positive	-	-	-	-	-	-	-	-	*Streptococcus spp.*

12a	Yellowish-white	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
12b	Colourless	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*
12c	Colourless	Cocci (clusters)	Positive	+	-	+	-	-	-	-	-	*Staphylococcus aureus*

KEY: a =>Nutrient agar, b =>McConkey agar, c =>Blood agar, + =>Positive, - => Negative, CA.=> Catalase, CI.=> Citrate, CO.=> Coagulase, IN.=> Indole, MR.=> Methyl-Red, VP.=> Voges-Proskauer, OX.=> Oxidase, UR.=> Urease.

Table 2: Frequency of Occurrence of Isolates by Patients Age and Sex

	Male		Female		Total	
Age (Year)	**No. of Samples**	**No. of Isolates (%)**	**No. of Samples**	**No. of Isolates (%)**	**No. of Samples**	**No. of Isolates (%)**
35-40	2	3 (25)	3	9 (37.5)	5	12 (33.3)
41-46	2	6 (50)	6	9 (37.5)	8	15 (41.7)
≥47	3	3 (25)	4	6 (25)	7	9 (25)
Total	7	12 (100)	13	24 (100)	20	36 (100)

Table3: Percentage Occurrence of Bacterial Isolates on Wound Sepsis

Isolates	**No. of Isolates**	**Percentage of Isolates**
Staphylococcus aureus	22	61.1
Streptococcus spp.	8	22.2
Pseudomonas spp.	6	16.7
Total	36	100

Table 4: Occurrence and Distribution of Isolates in Relation to Wound Type

Wound Type	**No. of Samples with Significant Growth (%)**	***Staphylococcus aureus***	***Pseudomonas spp.***	***Streptococcus spp.***	**Total No. of Isolates**
Burns	2 (16.7)	3	4	2	9
Post-Operative	3 (25)	7	-	1	8
Bruises	3 (25)	4	-	4	8
Abscess	4 (33.3)	8	2	1	11
Total	12 (100)	22	6	8	36

4.0. Discussion

Wound sepsis is common across all sex and age groups, and they cause disease burden both to the patient and the health services. Post-operative, bruises and abscess were the most common types of wounds in the cause of this research project.

From table 1, *Staphylococcus aureus* (61.1%) was the most predominant organism isolated from wound infection followed by *Streptococcus* spp. and *Pseudomonas* spp. this finding is also consistent with reports of similar studies conducted in various parts of the country such as Ibadan (Okesola & Kehinde 2008), Benin-city (Egbe, Omoregie, Igbarumah & Onemu 2011), Ekpoma (Isibor *et. al.,* 2008), Maidugri (Gadzama, Zailani, Abubakar & Bakari 2007), and elsewhere outside the country (Anbumani, Kaylan & Mallike 2006, Ohene, 1997). The high prevalence of *Staphylococcus aureus* infection may be because it is an endogenous source of infection. Infection with this organism may also be due to contamination from the environment, e.g. contamination of surgical instruments. With the disruption of natural skin barrier, *Staphylococcus aureus* which is a common bacterium on surfaces such as the human skin easily find their way into wounds.

Table 5: Antibiotic Sensitivity and Resistance Patterns of Isolated Gram Positive Organisms

	***Staphylococcus aureus* n=22**			***Streptococcus* spp. n=8**		
Antibiotic	**Sensitive (%)S**	**Resistance**	**Intermediate**	**Sensitive**	**Resistance**	**Intermediate**
Ciproflox	8 (36.4)	14 (63.6)	-	-	8(100)	
Gentamycin	8 (36.4)	12 (54.5)	2 (9.1)	-	8 (100)	-
Amoxil	2 (9.1)	19 (86.4)	1 (4.5)	-	8 (100)	-
Streptomycin	4 (18.2)	17 (77.3)	1 (4.5)	-	7 (87.5)	1 (12.5)
Rifampicin	13 (59.1)	7 (31.8)	2 (9.1)	3 (37.5)	4 (50)	1 (12.5)
Erythromycin	2 (9.1)	19 (86.4)	1 (4.5)	-	8 (100)	-
Levofloxacin	19 (86.4)	3 (13.6)	-	8 (100)	-	-

Table 6: Antibiotic Sensitivity and Resistance Patterns of Isolated Gram Negative Organism

***Pseudomonas* Spp. N=6**			
Antibiotic	**Sensitive**	**Resistance**	**Intermediate**
Ciproflox	2 (33.3)	3 (50)K	1 (16.7)
Tarivid	1 (16.7)	5 (83.3)	-
Streptomycin	3 (50)	3 (50)	-
Gentamycin	-	5 (83.3)	1 (16.7)

Streptococcus spp. (22.2%) was the second most prevalent bacterial agent isolated in this study. This could be based on the fact that *Streptococcus* can be isolated from nostrils and mouths of humans. Wounds can get infected when a patient pokes his nose and at the same time uses the same hand on the wound. Reports have also shown that overcrowding of patients in a ward, may contribute significantly to the high rate of cross infections in a Hospital setting (Shija, 1976).
Pseudomonas spp. (16.7%) was the least occurring bacteria in the course of this work, but was the commonest pathogen in burn wounds. Its high occurrence in wounds could be attributed to the anaerobic conditions of wounds.
The frequency of occurrence of isolates by age and sex was shown in Table 2. Males within the age range of 41-46 years had a frequency occurrence of 50% while the females within that same age range had 37.5%. This shows that the incidence of wound sepsis was more common in males than females. This is in agreement with studies done in different parts of Ethiopia (Gelaw, 2011; Biadglegne, Abera, Alem & Anagaw 2009; ; Tekie, 2008; Taye, 2005); Nigeria (Ohalete, Obi & Emeakoroha 2012; Amoran, Sogebi & Fatugase 2012) and India (Goswani, Trivedi & Goswami 2011) where male recorded more cases of wound sepsis than females.
The susceptibility testing of *Staphylococcus aureus* on ten selected commercial antibiotics showed that *Staphylococcus aureus* tend to be resistant to a wider spectrum of antibiotics. In this study, *Staphylococcus aureus* was highly resistant to Erythromycin (86.4%), Amoxil (86.4%), Streptomycin (77.3%) and Ciprofloxacin (63.6%). This finding is in agreement with the work of Gelaw,(2011); Bibi *et. al.,* (2012); Shamsuzzaman *et. al (*2003). The high resistance of *Staphylococcus aureus* to these antibiotics is because of the presence of a large mobile genetic element called Staphylococcal cassette chromosome, mec (SCCmec), that carries mecA genes that confere it with low binding ability to antibiotics. (Kurlenda, Grinholc, Krzyszton-Russjan & Wisniewska, 2009).
More so, *Pseudomonas* was resistant to commonly used antibiotics like Gentamycin (83.3%), Tarivid (83.3%), Streptomycin (50%), and Ciprofloxacin (50%). Ciprofloxacin has been stated to be the most potent oral drug available for the treatment of *Pseudomonas* infections. This report is in conformity with the result of other study in which Ciprofloxacin recorded the least resistant (6.2-24%) to *Pseudomonas* isolates from wound sepsis (Mulu, Kibri, Beyene & Damite 2012; Abraham & Wamisho 2009; Manyahi, 2012).

5.0. Conclusion.

Staphylococcus aureus was highly sensitive to Levofloxacin and Rifampicin. So, these antibiotics should be used in the treatment of wound sepsis. Their resistant to Amoxil, Erythromycin and Streptomycin denotes that these drugs cannot serve as a drug of choice in the treatment of wound infections.

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