Original Research Article

Antibiogram of Wound Isolates of Patients attending University of Port Harcourt Teaching Hospital

ABSTRACT

The risk of wound infection increases with the degree of contamination and it has been estimated that about 50% of wounds contaminated with bacteria become clinically infected. Profile of bacteria isolated from wound infections in patients attending University of Port Harcourt teaching Hospital (UPTH) were investigated. One hundred and eight wound isolates were collected from male and female patients and cultured on nutrient agar, Mac Conkey and blood agar. Based on morphological and biochemical characteristics of the isolates, *Staphylococcus aureus*44(40.75%) was the most prevalent in wound infection in the study area in both male and females followed by *Pseudomonas aeruginosa*28(25.9%), *Klebsiella* spp.14(13.0%), *Proteus* spp.10(9.3%) and*Escherichia coli* 12(11.1%). The antibiogram showed the presence of multi-drug resistant organisms. Imipenem (80%), Gentamycin (62%) clindamycin (30%), and levofloxacin (30%) should be considered as first line drug in the treatment of wound in the area as they were the most effective antibiotics. Prompt and timely treatment is therefore, recommended on the onset of wound infections.

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Keywords: Antibiogram, wound isolates, Teachings Hospital

INTRODUCTION

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Skin is the first line of defense and barrier against microbial invasion (Liu and Velazqez, 2018). The skin is a host to various transient bacteria contaminants. These are found in the openings of the hair follicles, sweat glands and sebaceous glands (Deurden*et al*., 2017). Any breach in the skin surface, whether accidental or surgical, provides an open door for bacterial infection (Dettenkofer*et al*., 2009). The skin is a protective barrier that shields the body from contamination by viruses, bacteria and toxins (Kirketerp *et al.,* 2018). Damage to the skin compromises the body's ability to fight off infections by providing an unobstructed route into the blood stream. According to Deurden*et al*. (2017), every individual carries a large resident microbial population on the skin surfaces, and in the openings of the hair follicles, sweat glands and sebaceous glands. *Propionibacterium, Corynebacterium, Pityrosporum* and *Staphylococcus* species have been reported to be part of the microbial population of the skin (Deurden*et al.,* 2017; Hsiao *et al*., 2011). The risk of wound infection increases with the degree of contamination and it has been estimated that about 50% of wounds contaminated with bacteria become clinically infected (Sahu *et al*., 2011). Hsiao *et al*. (2011) observed that *Staphylococcus aureus* accounts for 20-40% of hospital -acquired wound infection while Sahu *et al*. (2011) reported that *Pseudomonas aeruginosa* accounts for 5-15% of nosocomial wound infections. Other pathogens associated with nosocomial wound infections include *Staphylococcus* spp*, Klebsiella* spp*,Pseudomonas* spp*, Proteus* spp and *Escherichia coli* (Sahu *et al*., 2011). According to Sahu *et al.* (2011), nosocomial wound infection tends to be associated with bacteremia, septicaemia, shock and prolonged hospital stay in some patients.The aim of this study was to determine the antibiogram of wound isolates of patients at University of Port Harcourt Teaching Hospital.

MATERIALS AND METHODS

Description of Study Area

The study was conducted in the Medical Microbiology Laboratory of University of Port Harcourt Teaching Hospital(UPTH) located at East West Road Port Harcourt Rivers State Nigeria, a Tertiary Care Teaching and Research Facility.

Study Design

A cross-sectional descriptive analytical analysis was carried out in order to obtain a pure culture from the pathogenic bacteria isolates, followed by the antibiogram.

A total of 108 samples of wound isolates were studied within the periods of 3 months under aseptical condition and precaution in a standard medical microbiology laboratory.

Ethical approval was obtained from the Ethical research committee of the institution (UPTH), for the purpose of this research work.

Specimen Collection

The bacteria isolated from wound sample, was sourced from the Medical Microbiological laboratory of the University of Port Harcourt Teaching Hospital.

Wound isolates were transferred to the Medical Microbiological laboratory of the University of Port Harcourt Teaching Hospital and was subcultured as to obtain a pure culture.

Procedure for Collection of Pathogenic Bacteria

The culture media used in this study are nutrient agar, MacConkey agar, blood agar, and chocolate agar. Pathogenic bacteria already isolated from patients wound samples at the medical microbiology laboratory of the university of Port Harcourt teaching hospital where first inoculated using bijou bottle containing Solid media prepared in slant and transported to medical microbiology laboratory at Rivers State University, where it was subculture to obtain a pure culture isolate for gram staining and microscopy. After that biochemical test were carried out to confirm the pathogenic bacteria, before conducting the antibiogram of all the isolates.

Microscopical Examination of Wound Isolates

Wound isolates were smeared in a glass slide, heat fixed on a hot plate and stained with gram-stained reagents. Microscopical examination was carried out in in order to confirm and have a preliminary idea of the pathogenic bacteria isolates.

Morphological Characterization and Identification of Bacteria Isolates

The wound isolates were subcultured on nutrient agar, MacConkey agar, blood agar, chocolate agar and incubated at 37^{0C} for 24hrs, as to obtain a pure culture, for easy identification and characterization based on their colonial morphology and biochemical tests.

Antibiotic Susceptibility Testing

The method used here was pour plate method using Kirby-Bauer disc diffusion , which was done on Muller-hinton agar plate seeded with standardized suspension of purified isolates properly mixed in peptone water, and the mixture poured into the Muller-hinton agar plate and swirled to have an even distribution; after which the antibiotic single disc from different class of antibiotics was placed on the plate and allowed to incubate at 37^{0C} for 24hrs , and the Clearance zone diameters was examined and measured as to determine the antibiogram, in line with clinical laboratory and standard institute (CLSI) guidelines.

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RESULTS

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Table 1: The Overall Prevalence of Bacteria isolates from wound infection occurrence was in the order of;*Staphylococcus aureus* (40.7%), *Pseudomonas* (25.9%), *Klebsiella* (13.0%), *Escherichia coli* (11.1%) and *Proteus* spp (9.3%)

Table 1: Overall Prevalence of Bacteria isolates from wound infection

Isolates	Frequency	Percentage (%)
Pseudomonas aeruginosa	28	25.9
Proteus spp	10	9.3
Escherischia coli	12	11.1
Klebsiella spp	14	13.0
Staphylococcus aureus	44	40.7
Total	**108**	

Table 2: Showed distribution of wound isolates by Gender: Male patient was 56(51.9%) of the whole isolates whereas female patients were 52(48.1%).

In male, *Staphylococcus aureus* was frequently isolated 26(24.1) followed by pseudomonas aeruginosa 12(11.1%) while *Proteus spp* 4(3.7%) was the least isolated. The frequency of isolation in females was in the order of *Staphylococcus aureus* 18 (16.7%)>*Pseudomonas* 16(14.8%)>*Klebsiella* 8(7.4%)>*Proteus* 6(5.6%) and *E. coli* 4(3.7%).

Comparative results Mean±SD of wound Isolates in males and females showed no significant difference P>0.005 (P-value = 0.8722)

Table 2: Distribution of wound Isolates by Gender

Isolates	Male	Female
Pseudomonas aeruginosa	12(11.1%)	16(14.8%)
Proteus spp	4(3.7%)	6(5.6%)
Escherischia coli	8(7.4%)	4(3.7%)
Klebsiella spp	6(5.6%)	8(7.4%)
Staphylococcus aureus	26(24.1%)	18(16.7%)
Total	**56(51.9%)**	**52(48.1%)**

Comparative results Mean±SD of wound Isolates in males and females

Isolates	Male	Female	T-value	pvalue
Wound Isolates	11.20±8.786	10.40±6.229	0.1661	0.8722

P-value = 0.872

Table 3 showed Antibiogram: distribution of Antibiotics Susceptibility to wound isolates in the order of IPM(80), followed by GM(62), DA (32), LEV (30), CIP(30), E(28), AZM(27), FEP (27), CXM(2) and OX(2). Here, IPM(80) has the highest antibiotics Susceptibility distribution and OX has the least antibiotics Susceptibility distribution.

ANOVA results of mean ± SD of antibiotic of wound isolates, shows that within same row, values with different subscripts differs significantly at P<0.05, which implies that IPM, GM, are of same level of significant, LEV, AZM, CIP, CTX, FEP, DA and E are same level of significant and CXM also differs.

Table 3: Antibiogram: Distribution of Antibiotic Susceptibility of Wound Isolation

	IPM	**LEV**	**AZM**	**CIP**	**CTX**	**FEP**	**CXM**	**DA**	**E**	**GM**	**OX**
Staph. aureus	36(33.3%)	12(11.1%)	8(7.4%)	12(11.1%)	10(9.3%)	14(13.0%)	0(0%)	22(20.4%)	14(13.0%)	28(26.0%)	2(2.0%)
Escherischia coli	10(9.3%)	2(2.0%)	4(3.7%)	2(2.0%)	0(0%)	0(0%)	0(0%)	2(2.0%)	0(0%)	6(5.6%)	0(0%)
Klebsiella spp	12(11.1%)	6(5.6%)	6(5.6%)	4(3.7%)	4(3.7%)	2(2.0%)	0(0%)	2(2.0%)	4(3.7%)	12(11.1%)	0(0%)
Pseudomonas aeruginosa	14(13.0%)	8(7.4%)	8(7.4%)	10(9.3%)	6(5.6%)	10(9.3%)	2(2.0%)	4(3.7%)	8(7.4%)	12(11.1%)	0(0%)
Proteus spp	8(7.4%)	2(2.0%)	1(0.93%)	2(2.0%)	4(3.7%)	1(0.93%)	0(0%)	2(2.0%)	2(2.0%)	4(3.7%)	0(0%)
Total	**80**	**30**	**27**	**30**	**24**	**27**	**2**	**32**	**28**	**62**	**2**

Isolates:*Staph.aureus, Escherischia. coli, Klebsiella spp, Pseudomonas aeruginosa, Proteusspp*

ANOVA Results of Mean±SD of Antibiotic susceptibility of Wound Isolates

Antibiotics	**IPM**	**LEV**	**AZM**	**CIP**	**CTX**	**FEP**	**CXM**	**DA**	**E**	**GM**	**OX**	Fvalue	Pvalue
Susceptibility	16.0 ± 11.4^{a}	6.0 ± 4.2^{b}	5.40 ± 2.9^{b}	6.0 ± 4.6^{b}	4.8 ± 3.6^{b}	5.40 ± 6.2^{b}	0.40 ± 0.2^{c}	6.4 ± 3.7^{b}	5.6 ± 5.5^{b}	12.4 ± 9.4^{a}	0.4 ± 0.1^{c}	2.635	0.0131

Post Hoc: Within same row, values with different superscripts differ significantly at $p<0.05$.

Table 4, showed the antibiogram distribution of Antibiotics Resistant to all the isolates in the order of profile; OX(106), CIP(88), CTX(84), E(80), FEP(78), LEV(78), DA(76), GM(46), IPM(28) and CXM(16). Here, OX(106) has the highest antibiotics resistant distribution and CXM(16) has the least antibiotics resistant distribution.

ANOVA results of mean ± SD of antibiotic resistance of wound isolates shows no significant difference, P>0.05 (P-value = 0.104) in different antibiotics.

Table 4: Antibiogram: Distribution of Antibiotic Resistant of Wound Isolates

	1PM	LEV	AZM	CIP	CTX	FEP	CXM	DA	E	GM	OX
Staph. aureus	8(7.4)	32(29.6%)	36(33.3%)	32(29.6%)	34(31.5%)	30(27.8%)	0(0%)	2<(20.4%)	16(15.0%)	16(15.0)	44(38.9)
Escherischia coli	2(2.0%)	10(9.3%)	8(7.4%)	10(9.3%)	12(11.1%)	12(11.1%)	4(3.7%)	10(9.3%)	6(5.6%)	6(5.6)	12(11.1)
Klebsiella spp	2(2.0%)	8(7.4%)	8(7.4%)	10(9.3%)	10(9.3%)	10(9.3%)	0(0%)	12(11.1%)	2(2.0%)	2(2.0)	14(13.0)
Pseudomonas aeruginosa	14(13.0%)	20(18.5%)	20(18.5%)	18(16.7%)	22(20.4%)	18(16.7%)	12(11.1%)	24(22.2%)	16(15.0)	16(15.0)	28(25.9)
Proteus spp	2(2.0%)	8(7.4%)	8(7.4%)	8(7.4%)	6(5.6%)	8(7.4%)	0(0%)	8(7.4%)	6(5.6)	6(5.6)	10(9.3)
Total	28	78	80	88	84	78	16	76	80	46	106

Isolates: *Staph. aureus, Escherichia coli, Klebsiella* spp, *Pseudomonasaeruginosa, Proteus*spp.

ANOVA Results of Mean±SD of Antibiotic Resistance of Wound Isolates

Antibiotics	**IPM**	**LEV**	**AZM**	**CIP**	**CTX**	**FEP**	**CXM**	**DA**	**E**	**GM**	**OX**	Fvalue	Pvalue
Resistance	5.6±5.4	15.6±10.4	16.0±12.3	15.6±9.9	16.8±11.2	15.6± 8.8	3.2±5.2	14.8±6.8	9.2±6.4	9.2±6.4	21.6±14.3	1.726	0.1049
P-value						=						0.104	

DISCUSSION

The risk of wound infection increases with the degree of contamination and it has been estimated by Gosain in 2014 that about 50% of wounds contaminated with bacteria become clinically infected. Our findings revealed a variation in the distribution of pathogens among males (51.9%) and females (48.1%). This result was in agreement with the previous studied where the prevalence was higher in males (53.8%)than in female (46,2%) (Akinjogunla*et al*., 2019). However, the reason for this variation is unclear as most of the patients were not accident victims. The bacteria isolates obtained in the course of this study were *Staphylococcus aureus*, *Pseudomonasaeruginosa*, *Escherichia coli*, *Klebsiella* spp and *Proteus spp*. This result is in agreement with those of Thanni*et al*. (2013) from wound swabs in Ibadan and Swift*et al*. (2016) in Turkey.In addition to these five organisms, this is an indication that different bacteria contaminate and colonize wound infections, depending on the type and location of wound in the body. The most frequently isolated organism was *Staphylococcus aureus* (40.75%) followed by *Pseudomonas aeruginosa* (25.9%). This is in agreement with earlier works by Giacometti *et al*. (2000), Thanni*et al*. (2003) and Swift *et al*. (2016). They reported that the sources of most wound infections are endogenous flora of the patient's skin or mucous membrane. Imipenem (80%), gentamicin (62%), Clindamycin (32%), Levofloxacin (30%) and Ciprofloxacin (30%) were the most effective antibiotics in in vitro testing which was effective against all the pathogens. Similar results with aminoglycosides, betalactams and quinolones have been reported by other authors (Gupta *et al*., 2019; Goswami *et al*., 2011). Gupta *et al*. (2019) reported that quinolones were effective in treatment of wound infection caused by Gram negative bacteria. Oxacillin is most resistant to all the isolates. This may be due to wide and frequent use of these antibiotics (Campos *et al*., 2018), probably as a result of self-medication in the treatments of wounds even before visiting hospitals for treatment. Salu *et al*. (2011) and Hsiao *et al*. (2011), observed that *Staphylococcus aureus* account for 20% - 40% of hospital acquired wound infection, which is in line with the research work conducted. Another study by Trengove *et al*, 2011, report that Staphylococcus aureus has the highest frequency of 43% and Pseudomonas aeruginosa 7-33% in infected leg ulcers, which correlate with the findings conducted, including some Enterobacteriaceae such as *Escherichia coli*, *Klebsiella spp*, and *Proteus spp*.

Conclusion

This study revealed that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most frequent bacteria isolates from the samples studied, as this could have been acquired from the hospital, partly because most of the patients from whom this sample was collected are admitted in the hospital for at least for over one week and partly due to the fact that *Staphylococcus aureus* is one of the main flora on the skin and was the most predominant etiologic agent of wound infections. And also, there was presence of multiple drug resistant pathogens in the study area. There was a high level of resistance in Oxacillin which implies that is not suitable for treating cases of wound infection in this study area. As a matter of fact, culture and sensitivity testing of the isolates from wound swabs should be carried out prior to the administration of any treatment. This will help the clinicians to giveappropriate antibiotic selection and chemotherapeutic management of wound infections. However, imipenem, Gentamycin, Clindamycin, Levofloxacin, Ciprofloxacin, Erythromycin, Azithromycin should be considered as first line drug in the treatment of wound in this locality.

Recommendation

The researchers recommend multidisciplinary approach to wound management, which should involve the Medical Laboratory Scientists, environmental microbiology, Clinicians, Infection control officers and pharmacist. Laboratory services should be strengthened at local and regional levels for effective antimicrobial susceptibility surveillance.

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