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BACTERIOLOGY OF WOUND INFECTIONS

G. E. Chukwuma[1] and T. J. T. Princewill[2]

[1]*Department of Microbiology, University of Port Harcourt, Port Harcourt, Nigeria.*
[2] *Formally, Dean of the Faculty of Biological Science, University of Port Harcourt, Port Harcourt, Nigeria*.

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ABSTRACT
The nature of the bacterial pathogens associated with wound infections was bacteriologically evaluated. Out of a total of eighty – one (81) swab samples from wound analysed, three samples were found negative, ie, no growth, representing a frequency of 4%. Seventy – eight (78) samples were found to be positive which represented a frequency of 96%. Eight isolates resulted from four samples that showed polymicrobial growth, while there were seventy – four isolates from monomicrobial growth. Among these positive isolates, thirty – five (35) were gram positives and forty – seven (47) were gram negatives representing frequencies of 43% and 57% respectively. Biochemical tests classified these bacteria into specie – levels that showed Staphylococcus species with highest incidence of thirty – five (35) isolates that represented a frequency of 44%, followed by Pseudomonas species with nineteen (19) isolates that represented a frequency of 24%. Proteus species with fourteen (14) isolates ranked third with a frequency of 18%. Klebsiella and Escherichia species with nine (9) and five (5) isolates had frequencies of 11.5% and 6.4% respectively. Most of these organisms showed more than 50% resistance to a greater number of the antimicrobial agents tested. The resistance rate of more than 50% by most of these organisms poses great challenge to medical care, and will adversely affect choice of treatment for severe infections, therefore , this calls for better and proper prophylactic measures, such as cleanliness, carefulness, as well as good diets.

Key words: Bacteriology, Wound isolates, Antimicrobial agents and Susceptibility.

INTRODUCTION
Microorganisms may either play a role as commensals or may constitute a serious threat to human life by causing infectious diseases. The genesis of these diseases depends on a number of factors, such as the defence mechanisms of the body, the numbers and virulence of the microorganisms,etc.(Willey et. al., 2014 A).

The microorganisms found in wounds may belong to the normal bacterial flora in the environment – the gram positive and gram negative microorganisms on the skin and in the alimentary tract. Fungi and viruses may sometimes be implicated in wound infections. In some centres, bacteriological analysis of wound infections is limited to six main genera and these are *Staphylococcus, Streptococcus, Proteus, Pseudomonas, Escherichia* and *Klebsiella.*

Efforts have been made by earlier researchers to isolate and characterize the causative organisms and treat the menace of these micro-organisms in our hospital wards. Despite these efforts, many patients in our

hospital wards still present with many life threatening and antibiotic resistant wound infections. The objective of this research is to evaluate bacteriologically the nature of the bacterial pathogens associated with wound infections sampled at the University of Port Harcourt Teaching Hospital.

MATERIALS AND METHODS

Source of Samples

With a letter of introduction from the Department of Microbiology, University of Port Harcourt, eighty – one (81) wound samples were obtained from the Diagnostic Microbiology Department, University of Port Harcourt Teaching Hospital**.**

Collection of Specimens

Swabs were taken from patients with wounds admitted in the hospital wards. The collected samples were immediately plated out and incubated aerobically, anaerobically and microaerophilically or stored in the refrigerator (4^0C) for a few hours when the media were not ready or when culture had to be done in school laboratory. Specimen collection, transport, and processing were carried out using conventional methods (Willey et. al., 2014 A; Forbes et. al., 2007 A; Cruickshank etal., 1980)

Cultural, Morphological and Biochemical Characterization

The appropriate media were prepared in accordance with the Oxoidmanufacturer's instructions. The wound samples were inoculated into Blood agar, MacConkey agar and CLED plates, using streak plate method. In some cases, the Blood agar is supplemented with brain heart infusion and activated charcoal in other to recover gram positive and gram negative bacteria in the presence of antimicrobial agents. MacConkey media helps to differentiate between lactose fermenters and non – lactose fermenters. CLED being an electrolyte deficiency media prevents swarming of *proteus*spp and is a differential media. And the inoculated plates were incubated aerobically, and microaerophilically for 24 hours and 48 hours respectively at 37^0C. Anaerobic cultivation was also set up. All the isolates (microbial organisms) were identified by their cultural, microscopic and biochemical characteristics using standard methods (Willey et. al., 2014 A; Forbes et. al., 2007 A; Cruickshank et.al., 1980)

Drug Sensitivity Test

Antibiotic disk (Multi disk, Oxoid, England Codes 1789 E and 1788 E) were used for gram positive and gram negative respectively. This was commercially produced by impregnating known concentrations of different antimicrobial drugs on absorbent paper disks. The antimicrobial drugs used in this experiment are listed below in table 1 and were used according to the gram reaction of the organisms. The antibiotic disks were normally stored in the fridge at 4^0C.

The identified organisms were plated out on the Mueller – Hinton agar plates. The compound disks were removed with a pair of sterile forceps and placed on the surface (centre) of the Mueller – Hinton agar culture. The plates containing the disks were incubated for 24 hours at a temperature of 37^0C. The sensitivity patterns were recorded and compared with available standards (Muluetal., 2012; Kibret and Abera, 2011; Youmanetal., 1980). Drugs with zones of inhibition whose diameters were below the reported standards (0 – 1mm), were classed as those to which the organisms were resistant, while those with zones of

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inhibition showing the same as or greater than those shown by the standards were grouped as those to which the organisms, were sensitive (>1mm – 4mm for low sensitivity; >4mm – 8mm for moderate sensitivity; >8mm – 15mm for high sensitivity). This interpretation of result was according to Nationnal Committee for Clinical Laboratory Standards (NCCLS).

RESULTS

Bacterial Isolates With Respect To the Samples

Eighty – one wound-swab samples analysed bacteriologically, presented both gram positive and gram negative organisms with the exception of three samples that showed no growth. Out of eighty – one (81) samples, positive growth was observed in seventy – eight (78) samples representing a ninety – six percentage (96%) frequency. Out of these eight – one (81) samples, 74 samples showed monomicrobial growth, while four (4) showed polymicrobial growth. And three (3) samples showed no growth. In all, a total of thirty – five (35) isolates were gram positive and forty –seven (47) were gram negative while there were no growth in samples 14, 22 and 80. An intensed red colour (Methyl red test) was noticed in some isolates which persisted much longer while some samples had faint red colours that disappeared much faster. And there were other positive results with intermediate colouration between these deep red and faint red colourations. From the result of carbohydrate fermentation test, *Pseudomonas* species had a very faint and late positive colour for glucose and negative for other sugars used. This is in line with many literatures and books (Carroll et al., 2016; Willey et. al., 2014 A; Forbes et. al., 2007 A; Cruickshank Vol. ll, 1980).

The bacteria were properly characterized and their distribution among different wound types were shown in table 2.Out of eighty two (82) bacterial isolates, 15 (18.3%) bacterial isolates were isolated from osteomyelitis while 11 (13.4%) were from burn's infections. Five (6.1%) bacterial isolates were from diabetic ulcers. Majority of the isolates, 51 in number, representing 62.2% were isolated from surgical cases, leg and pressure ulcers, obstetrics and gynaecologicalcases and urological cases. The frequency of isolation of bacteria from the different wounds is shown in table 3. The susceptibility of the microbial isolates were also observed and were reported in tables 4 and 5. Most of the organisms were resistant to the antimicrobial agents tested.None of the gram negative bacteria was sensitive to Compound Sulphonamide (S3). In general, the gram positive bacteria were more sensitive to the antimicrobial agents tested.

Table 1: Antimicrobial Drugs (Disk) Used

Drug	Concentration	Codes
Gram Positives (Code 1789 E):		
Ampicillin	2 mcg	Amp
Chloramphenicol	10 mcg	C
Cloxacillin	5 mcg	OB
Erythromycin	10 mcg	E
Penicillin	1.5 iu	P
Streptomycin	10 mcg	S
Tetracycline	10 mcg	TE
Co – trimoxazole	25 mcg	Sxt
Gram negatives (Code 1788 E)		
ColistinSulphate	0 mcg	CT
Nalidixic Acid	30 mcg	NA
Nitrofurantoin	200 mcg	F
Compound Sulphonamide	300 mcg	S3
Streptomycin	25 mcg	S
Tetracycline	50 mcg	TE
Co – trimoxazole	25 mcg	Sxt
Ampicillin	25 mcg	Amp

Table 2: Distribution of Bacterial Isolates among Different Wound Types.

Wound type	Frequency of isolates (%)					
	1	**2**	**3**	**4**	**5**	**Total**
Osteomyelitis	10 (67)	0 (0)	4 (27)	1 (6)	0 (0)	15 (18)
Burns	2 (18)	7 (64)	0 (0)	1 (9)	1 (9)	11 (13)
Diabetes	1 (20)	1 (20)	1 (20)	2 (40)	0 (0)	5 (6)
Surgical*,etc.	22 (43)	11 (21)	9 (18)	5 (10)	4 (8)	51 (62)
Total	35	19	14	9	5	82

Key: **1** =*Staphylococcus aureus,* **2**=*Pseudomonas* spp,**3** =*Proteus* spp,**4** =*Klebsiella*spp, **5** = *Escherichia coli.* Figures in brackets are percentages of the microorganisms in different wound types. Surgical* = surgical cases, leg and pressure ulcers, obstetrics and gynaecological cases and urological cases.

Table 3: Frequency of Isolation of Bacteria from Different Wound Types

Woud type	Positive Growth		No	Total
	Monomicrobial	Polymicrobial	growth	samples
Osteomyelitis	11	4	2	17
Burns	11	0	0	11
Diabetes	5	0	0	5
Surgical, etc.	47	4	1	52
Total	74	8	3	85

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The percentage sensitivity and resistance of the bacterial isolates to the antimicrobial agents are shown below.

Table 4: Susceptibility Pattern of Gram Positive Isolates

		Number Sensitive To Antimicrobial Agents(%)							
GPI	**Interpretation**	**S**	**C**	**E**	**OB**	**TE**	**SXT**	**P**	**AMP**
S. aureus	Sensitive	8(23)	11(31)	6(17)	19(56)	6(17)	17(49)	1(3)	4(11)
	Resistant	27(77)	24(69)	29(83)	16(46)	29(83)	18(51)	34(97)	31(89)

Key for antimicrobials - as shown in table 1. Figures in brackets are percentages of test isolates sensitive to the antimicrobial agents tested; GPI =Gram positive isolates.

Table 5: Susceptibility Pattern of Gram Negative Isolates

		Number Sensitive To Antimicrobial Agents(%)							
GNI	**Interpretation**	**S**	**TE**	**SXT**	**AMP**	**S3**	**NA**	**F**	**CT**
Pseudomonas spp	Sensitive	6(32)	0(0)	0(0)	0(0)	0(0)	4(21)	7(37)	4(21)
	Resistant	13(68)	19(100)	19(100)	19(100)	19(100)	15(79)	12(63)	15(70)
Proteus spp	Sensitive	7(50)	1(7)	1(7)	0(0)	0(0)	5(36)	4(29)	6(43)
	Resistant	7(50)	13(93)	13(93)	14(100)	14(100)	9(64)	10(71)	8(57)
Klebsiellaspp	Sensitive	6(86)	3(4)	4(57)	1(14)	0(0)	4(57)	5(71)	6(86)
	Resistant	1(14)	4(57)	3(43)	6(86)	7(100)	3(43)	2(29)	1(14)
E. coli	Sensitive	4(57)	2(29)	2(29)	2(29)	0(0)	6(86)	5(71)	6(86)
	Resistant	3(43)	5(71)	5(71)	5(71)	7(100)	1(14)	2(29)	1(14)

Key for antimicrobials - as shown in table 1. Figures in brackets are percentages of test isolates sensitive and resistant to the antimicrobial agents tested; GNI =Gram negative isolates.

DISCUSSION

Most of the results obtained were in accordance to the cultural and biochemical characteristics of the organisms as obtained in books and literatures –Willey et. al., 2014 A; Forbes et. al., 2007 A; Cruickshank Vol. II, 1980. On the nature of the organisms isolated, the results showed a greater prevalence of gram negative organisms. The predominance of gram negative bacteria in the aetiology of wound infections has been recognized and considered to be related to various factors as reported by Willey et al., 2014 B and Gedebou et. al., 1983. The authors attributed this mainly to the widespread and intensive use of antibiotics as well as the new and complex surgical operations and procedures.

However, the result expressed at specie level showed *Staphyloccocusaureus* (gram positive) as having the highest prevalence. This is in line with reports from some of the literatures by Olson and Horswill (2013), Vincent and Coleman (2008). *Staphylococcus aureus* with the highest incidence showed a frequency of 43%, followed by *Pseudomonas*spp, *Proteus* spp, *Klebsiella*spp, and *Escherichia coli* with frequencies of 23%, 17%, 11%, 6% respectively. The bacteria types isolated varied from researchers to researchers – some have gram negative bacteria as predominant while others have gram positive bacteria. This simply shows that there is no definite rule to the bacteria types isolated in any study, but it depends on the environment concerned, as many studies have shown. Our finding that *Staphyloccocusaureus* was the most frequent isolate in osteomyelitis (67%) was similar to the work of Olson and Horswill, 2013, Vincent and Coleman, 2008.

Our results showed that only aerobic and facultative organisms were isolated. Our inability to isolate any anaerobic organisms could be attributed to the use of tetanus toxoid and to other factors such as the method of specimen collection, preservation, time factor, cultural methods (Willey et. al., 2014 A; Forbes et. al., 2007 A; Finegold, 1980; Cruickshank et. al, 1980). Or it could be attributed to low incidence of these organisms within the area studied. Most of these anaerobic organisms (anaerobic Streptococci, Bacterioides, etc) are found in deep wounds, so in other not to miss these important organisms, proper and standard method of sample collection should be adopted, while surface, superficial collection of samples should be avoided. Also , transport medium has been advocated for conveying specimen from wards, hospitals to the laboratories (Willey et. al., 2014 A; Forbes et. al., 2007 A; Cruickshank et.al . 1980; Finegold, 1980). The introduction of tetanus toxoid is an important factor which has contributed immensely to the reduction of Closthridial infection.

The data obtained in the sensitivity and resistance rate of the microorganisms isolated to the antimicrobial agents showed that the rates of susceptibilities of nearly all the different bacteria isolates to the antibiotics that are prescribed in the hospitals were very low and is similar to the work of Mulu et. al., 2012; Gedebou et al , 1983. There is need to control antibiotic utilization in our hospitals and this can be done by tailoring antibiotic prescriptions to microbiological results and terminating same in most instance after one week to one and half weeks (Paul, 2006). According to literatures and manuals (Willey et. al., 2014 B; Carroll et. al., 2016; Forbes et. al., 2007 B, Meakins et. al, 1980), there is an

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additional concept that the resistance is transferred from one organism to another. The authors reported that gram-negative organisms may transfer resistance by sexual conjugation and the movement of a plasmid from one organism to another. In gram-positive organisms, antibiotic resistance may also be plasmid mediated and transferable. These transferable resistance determinants (factors) are referred to as episomes(Willey et. al., 2014 B; Carroll et. al., 2016; Wilson and Miles, 1975). These episomes like plasmids are genetic complement of a cell carried on an extrachromosomal element. *Pseudomonas* species were found to be the most resistant organisms, having 100% resistance to many drugs tested such as Ampicillin, compound sulphonamide and tetracycline. This could be attributed to the extrachromosomal element carried by most organisms. Such extrachromosomal element probably is the type which contain all the genes capable of synthesizing enzymes that could destroy all the drugs concerned. Thus, some antibiotic resistance in some bacteria like *Staphylococcus aureus*and *E. coli* is usually associated with the production of B – lactamase (enzyme) which destroy these antibiotics (Willey et. al., 2014 B, Forbes, et. al., 2007 B). Our finding of some doubtful zones of inhibition with peripherial striated margins on some samples could be as a result of the bacteriostatic nature of the agents concerned, by the time the drug effect could diffuse to the peripherial area, a considerable colony had already been formed and the drug's effect could only be momentary, thereby no lysis of cells, whereas, in the case of bactericidal antibiotics the developed colonies would have been wiped out entirely. The resistant organisms might have carried resistant factors – the episomes (Willey et. al., 2014 B; Carroll et. al., 2016; Wilson and Miles, 1975). The authors likened this deduction to what is obtained in abortive transduction. Since these resistant factors can alternate between extra chromosomal and chromosomal locations; it could be reasoned that since it is the inducer of resistance to a cell, any daughter cell that don't receive a portion of it due to extra-chromosomal location or other binary difficulty during cell division would become sensitive. This means that those that retain them chromosomally are resistant. It is necessary to note that Nalidixic acid and Nitrofurantoin, though were sensitive to some micro-organisms, are not used therapeutically for isolates from wound. This is because they are bladder or urinary disinfectants and as such are not used for systemic and tissue infections. Compound sulphonamide (S3) was not effective to any of the isolates but became effective against some micro-organisms when in combined form as septrin (co-trimoxazole) as shown in table 5. This is because the drugs (sulphamethoxazole and trimethoprim) that make up septrin, possess respective degree of antibacterial activity that act against different pathways in the bacterial metabolism as reported by Ehrlich, 1913, cited by Mackie and MacCartney, 1983. This is also documented by Guozhi et. al., 2016, D Byron, 2016,, Willey et. al., 2014 B,Carroll et. al., 2016, in which the authors reported that combined therapy is best carried out with therapeutic agents which attack entirely different chemoreceptors in the parasite – a simultaneous and varied attack on the parasite in accordance with the military maxim, march apart but fight combined.

Infection in general is not possible if the natural barriers are well maintained. This goes in accordance with Willey et. al., 2014

B; Carroll et. al., 2016; Meakins et. al, 1980, in which they reported that in and on most epithelial surfaces, there are mechanical, chemical and bacteriologic barriers to colonization, that prevent lodgement and subsequent development of bacterial infection. The resistance rates of more than 50%, in almost all the drugs and up to 100% in some, is a cause for concern, for the fact that this will adversely affect the choice of treatment for severe illnesses. This calls for a better and proper prophylactic measures such as cleanliness, hand washing in and out of all hospital departments, use of antiseptics, one pair of disposable hand grove and niddle for each patient, discouraging use of re usablesterilizable needles,etc. As most of these organisms especially in surgical cases, can be as a result of cross-infection, cleanlinesswill be a watch – word for surgeons, physicians, paramedical staff and the patients themselves. Hand washing at beginning and end of every examination and in – between surgical procedures will be a watch – word for surgeons, physicians and paramedical staff This goes in line to Bloomfield etal., 2007, Meakinsetal, 1980; in which the authors reported that the most effective control measure of cross – infection is still handwashing before and after every patient contact, in contrast to the use of antibiotics as prophylactic measures which may induce resistance pattern. Injection of tetanus toxoid when one sustains injuries especially in the laboratories goes a long way to reduce infection rates particularly those of closthridum. Other preventable measures such as carefulness and respect among road users and those engaged in accident – prone businesses are also recommended. When all these measures have been exhausted, the unavoidable cases of resistant infections can be approached using combined drug therapy as reported by Ehrlich, 1913, cited by Guozhi et. al., 2016, D Byron, 2016, Mackie and MacCartney, 1983, Willey et. al., 2014 B. Lastly, the nutritional status of the patient must be highly priced for effective and prompt recovery to be attained.

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