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In vitro Antimicrobial Susceptibility Testing of Bacterial Isolates Causing Wound Infection in a Tertiary Care Hospital, Port Harcourt, Nigeria

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ABSTRACT

A successful treatment of wound infection is determined by the proper identification and evaluation of the varied types of microorganisms that colonize the wound surface. Such evaluation will include an antimicrobial susceptibility profiling of the invading pathogen(s) in order to implement an effective and pathogen specific treatment. The antimicrobial susceptibility profile of the bacterial species isolated from wound infections was tested to provide basis for their prudent use as antimicrobials. The standard method of antibiotic sensitivity testing with single antibiotic disc was employed in the analysis. The bacterial isolates tested were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The organisms were isolated from samples collected from patients at different wards at the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. The number and percentage occurrence of each individual bacterium encountered varied among the isolates. *Pseudomonas aeruginosa* has the highest frequency of occurrence of 48.6% accounting for 36 of the 74 bacterial isolates. This was followed by S*taphylococcus aureus* and *Escherichia coli* accounting for 17 (23.0%) and 11 (14.9%) respectively. *Klebsiella pneumoniae* was the least prevalent bacteria species accounting for 10 (13.5%) of the total bacterial count. The study shows imipenem as the most potent antimicrobial agent against the isolates tested. The isolates were moderately sensitive to gentamicin, ciprofloxacin and levofloxacin, but highly resistance to ceftazidime, erythromycin, sulphamethoxazole/trimethoprim, cefepime, amoxicillin/clavulanic acid and aztreonam. The study further reiterates the need for prudent use and control of antimicrobials.

Keywords: Antibiotics, susceptibility, resistance, wound infection, bacterial isolates.

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INTRODUCTION

One of the key functions of a normal or intact skin as earlier indicated is to ensure that there no proliferation of undesirable microbes (that can cause a disease) on the skin surface and the underlying tissues. [1-2] In the event of a breakage in the continuity of the skin a

disease condition may arise obviously due to the presence of undesirable microbes. [3-5] These invading organisms could be aerobic or anaerobic organisms varying in time and location of infections. [6] Increasingly, newer pathogens are being identified in different kinds of wound infections and these organisms as previously reported may be difficult to identify drugs. [7] In some way become resistant to most of the available antimicrobial drugs and may as well contribute to the nonhealing of wounds that do not exhibit clinical signs of infection. [3, 7] In managing wound infection, several factors would have to be considered collectively to assess the probability of infection. Earlier reports have considered factors such as type of wound, location of the wound, microbial density, microbial type. [3, 8-9] Other important factors include certain disease conditions such as cancer, diabetes, poor blood supply to the wound and host immune response. [3]

Majority of the wound's infections reported in several literatures show *Staphylococcus aureus* as the causative organism. [2, 7, 10-11] *Pseudomonas aeruginosa* seems to account for 5-15% of nosocomial infections with infection mainly following burns and surgery. [2, 10] *Escherichia coli* is seen as a common inhabitant of the human and animal gut, but can also be found in water, soil, vegetation, wounds, otitis media and other complications in humans. [7, 12] Earlier report affirms the occurrence of an infection when virulence factor(s) is expressed by one or more microorganisms in a wound to the extent that it out compete the host natural immune system and subsequently invades and disseminates microorganisms in viable tissue and so provokes a series of local and systemic host responses. [3] The responses may give rise to cellulitis around the wound following a purulent discharge or painful spreading erythema. [3] The wound may also progress to an infected state depending on the state of the invading microbe as well as the host. [3]

Previous report shows millions of people suffering from nonhealing or wounds complicated by infections every year despite advances in infection control. [13] These are serious problems in most developing countries including Nigeria, where preventable and curable wound infections have become contaminated with virulent and resistant strains. [10, 13-14] This is further complicated by increasing prevalence of multidrug resistant pathogens acquired from either health care setting environment, health care personnel, or inpatients and the misuse of antibiotics by the public. [13, 15-16] Undoubtedly, patient suffers some form of inconveniences including burden on financial resources and the increasing requirement for cost-effective management within the healthcare system. [13, 17-19] It is therefore important to identify those wounds in which healing is impaired as a result of infection or heavy bacterial burden and in which systemic or topical antimicrobial treatment will be of benefit. [11] Knowledge of the causative agents and choice of treatment are essential factors for adequate institution of appropriate antimicrobial therapy. As previously reported, antibiotic sensitivity testing should be carried out side by side as a confirmatory diagnosis to determine the infection and the causative agent. This study was conducted to characterize the bacterial isolates from wound infections at the University of Port Harcourt Teaching Hospital (UPTH) Nigeria with the view of determining their susceptibility pattern to selected antibiotics.

Table 1: Number of samples collected from the different wards in the UPTH

Ward	No. of samples collected
Male Orthopedic ward	11
Female Orthopedic ward	6
Male Medical ward	4
Male Accident and Emergency ward	5
Female Accident and Emergency ward	1
Male Surgical ward	3
Female Surgical ward	3
Male Internal medicine ward	2
Female Internal medicine ward	6
Male General Out-Patient Department (GOPD) ward	2
Female General Out-Patient Department (GOPD) ward	2
Male Burns Unit	5
Total	50

MATERIALS AND METHODS

Samples Collection

A total of fifty (50) infected wound samples were collected from male and female patients at different wards namely; orthopedic, surgery, medical, accident and emergency, general outpatient department (GOPD) dressing room and the burns unit of the University of Port Harcourt Teaching Hospital (Table 1). The samples were collected using sterile swab sticks rubbed firmly over the patients wound surface using parallel strokes with slow rotation of the swab and then repeating this at right angles to the first strokes as previously described. [2, 13] The samples were labeled and transferred aseptically to pharmaceutical microbiology laboratory, University of Port Harcourt for examination. [3, 20-21]

Ethical approval

This study was approved by the research and ethics committee of the University of Port Harcourt Teaching Hospital.

Inoculation technique

At the Laboratory, the swab sticks tips were cut off aseptically using a flamed and cooled pair of scissors into bijou bottles containing inoculating nutrient broth. The bijou bottles now containing samples were then incubated in Memmert incubator (Germany) at 37°C for up to 24 hours to allow for microbial growth.

Isolation of pure culture

The streak plate method was used in isolating pure culture of each sample. [2] The streak plate method is a simple but rapid method used in diluting the sample

by mechanical means. A sterile loop was dipped into the bottle containing the nutrient broth and swab stick inoculated 24 hours before, and then a loopful of the broth culture was streaked across the dried agar surface of different agar media: Mannitol salt agar (Lab M, England), MacConkey agar (Lab M, England) and Cetrimide nutrient agar (Lab M, England). [13, 22] The agar plate was then incubated in Memmert incubator (Germany) for another 24 hours at 37°C to allow for microbial growth. Plates showing distinct colonies were selected to be used for identification and characterization. From purified plates, colonies were collected to be maintained in slants made from nutrient agar for future use. Each slant was carefully labeled and sub-cultured by streaking in successive areas of a freshly dried agar plates and incubated at 37°C for 24 hours. Purity of each isolate was confirmed by their colonial morphology and Gram staining. [23-25] All samples from the point of collection to identification and characterization were carefully labeled to prevent mix ups and subsequent error in result.

Identification of isolates

The isolated organisms were identified by their cultural, gram reaction, microscopic and biochemical reactions using standard methods. [23-25]

Antibiotic susceptibility testing

The susceptibility patterns of the isolated organisms were determined through tests using a wide range of antibiotics comprising of single antibiotics discs (Oxoid, UK) impregnated with known concentration of antimicrobial agent. The discs were placed on plates of solidified Mueller Hinton agar (Lab M, England) uniformly inoculated with the test organisms and allowed to diffuse for 15 minutes before incubation. [20, 26] A list of the antibiotics used for the different microorganisms include, *Pseudomonas aeruginosa*– Ceftazidime (30µg), Gentamicin (30µg), Piperacillin / Tazobactam (110µg), Cefepime (30µg), Imipenem (10µg), Ticarcillin (85µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Aztreonam (30µg). *Escherichia coli* / *Klebsiella* spp - Ceftazidime (30µg), Gentamicin (30µg), Imipenem (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Aztreonam (30µg), Amoxicillin / Clavulanic acid (30µg), Sulfamethoxazole-Trimethoprim (25µg/1.25 mg), Polymyxin B (300 units), Erythromycin (15µg). *Staphylococcus aureus* - Ceftazidime (30µg), Oxacillin (1µg), Vancomycin (30µg), Gentamicin (30µg), Erythromycin (15µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Sulfamethoxazole-Trimethoprim (25µg). The impregnated plates were incubated for 24 hours at 37°C and observed to see whether there was any zone of inhibition. Zone of inhibition was interpreted based on the Clinical Laboratory Science Institute (CLSI) standards. [20, 26]

RESULTS

Number and percentage occurrence of bacterial isolates

Fifty samples were collected from patients with varying degree of wound infections between March and April 2015 from twelve (12) different wards at the University of Port Harcourt Teaching Hospital (UPTH). Out of fifty wound samples collected majority of the patients were males 32 (64%) as opposed to 18 (36%) for females (Table 1). The number and percentage occurrence of each individual bacterium encountered are presented in Table 2. The microbial isolate *P. aeruginosa* was seen to have the highest frequency of occurrence of 48.6% accounting for 36 of the 74 bacteria isolates. This was followed by S. *aureus* and *E. coli* accounting for 23.0% and 14.9% (or 17 and 11) of the bacterial isolates respectively. *K. pneumoniae* was the least prevalent bacterium accounting for 10 (13.5%) of the total bacterial isolate.

Table 2: Number and percentage occurrence of each bacteria species isolated in the 50 samples.

Organism	No of occurrence	Percentage occurrence
Pseudomonas aeruginosa	36	48.6
Staphylococcus aureus	17	23.0
Escherichia coli	11	14.9
Klebsiella pneumoniae	10	13.5
Total	**74**	**100**

Table 3: Pattern of Poly-microbial growth.

Organisms occurring together	No of occurrence	Percentage occurrence
P. aeruginosa/ *S. aureus*	8	42.0
P. aeruginosa/ *K. pneumoniae*	4	21.1
P. aeruginosa/ *E. coli*	4	21.1
P. aeruginosa/ *K. pneumoniae*/ *S. aureus*	2	10.5
P. aeruginosa/ *S. aureus*/ *E. coli*	1	5.30
Total	**19**	**100**

Pattern of poly-microbial growth

Microbial growth during this study showed both poly-microbial and mono-microbial pattern. Some samples showed a single bacterium while others were either two or more bacteria and the results are presented in Table 3.

Antimicrobial susceptibility pattern of bacterial isolates

Results of the antibiotic susceptibility testing were interpreted as resistant, intermediate or susceptible based on guidelines on antimicrobial susceptibility testing for conventional drugs by the National Committee for Clinical Laboratory Standards (NCCLS) now known as Clinical Laboratory Standards Institute (CLSI). [26] All isolates showed high frequency of resistance to cefepime and ceftazidime, and high susceptibility to imipenem; *K. pneumoniae* and *E. coli* showed high resistance to aztreonam and amoxicillin-clavulanic acid. As presented in Fig. 1, *P. aeruginosa* showed 100% resistance to ceftazidime like all the other isolates, and 100% resistance to cefepime, aztreonam, piperacillin-tazobactam, ticarcillin but susceptible to imipenem, variable susceptibility pattern to gentamicin, and 50% susceptibility to ciprofloxacin and levofloxacin.

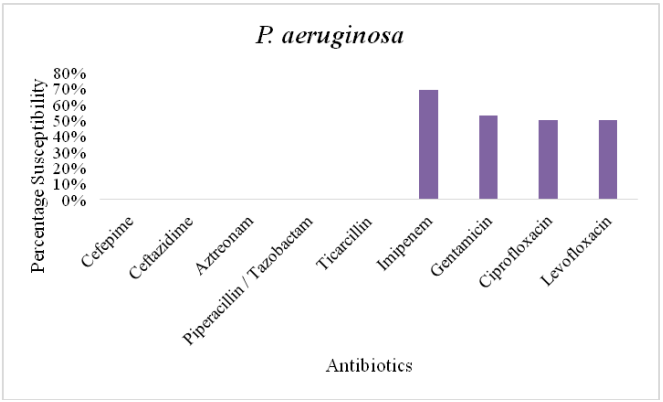

Fig. 1: Susceptibility pattern of *Pseudomonas aeruginosa*

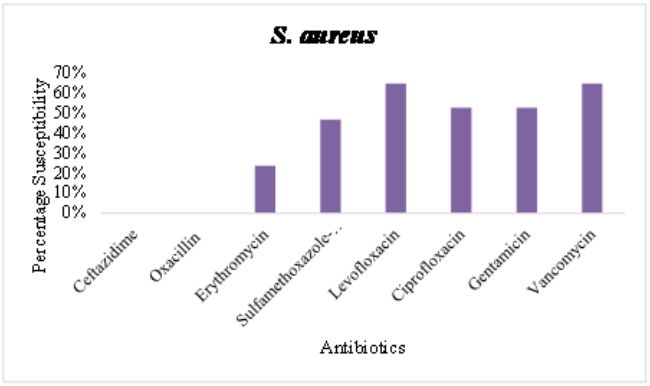

Fig. 2: Susceptibility pattern of *Staphylococcus aureus*

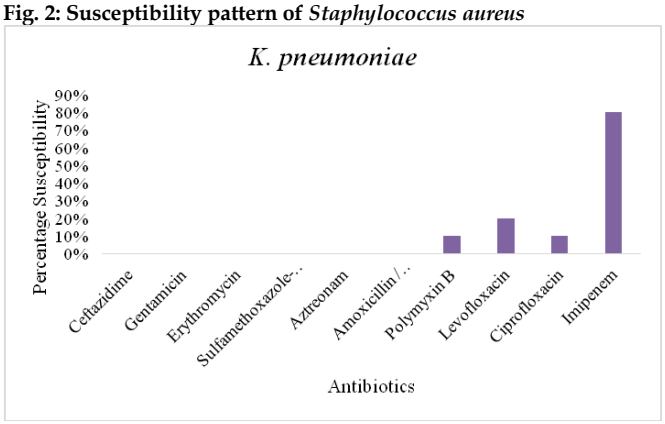

Fig. 3: Susceptibility pattern of *Klebsiella pneumoniae*

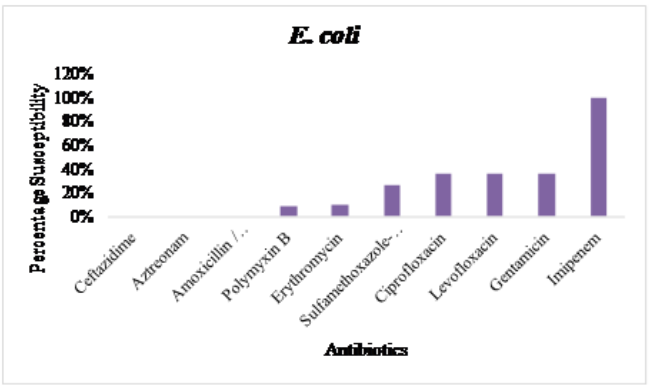

Fig. 4: Susceptibility pattern of *Escherichia coli*

Resistance and susceptibility pattern of *Staphylococcus aureus*

Staphylococcus aureus showed 100% resistance to ceftazidime, and oxacillin, 76% resistance to erythromycin, 53% resistance to Sulfamethoxazole-Trimethoprim, and a slightly above average susceptibility pattern to ciprofloxacin, gentamicin and levofloxacin of 53%, 53% and 65% respectively (Fig. 2).

Resistance and susceptibility pattern of *Klebsiella pneumoniae*

Resistance and susceptibility pattern of *Klebsiella pneumoniae* presented in Fig. 3 showed multiple drug resistance, with 100% resistance to ceftazidime, gentamicin, erythromycin, sulfamethoxazole-trimethoprim, aztreonam, amoxicillin-clavulanic acid, 90% resistance to polymixin B and ciprofloxacin, 80% resistance to levofloxacin and a high susceptibility (80%) to imipenem.

Resistance and susceptibility pattern of *Escherichia coli*

Escherichia coli also showed multiple drug resistance, with 100 % resistance to ceftazidime, aztreonam, amoxicillin-clavulanic acid, 91% resistance to polymixin B and erythromycin, 73% resistance to sulfamethoxazole-trimethoprim, 64% resistance to ciprofloxacin, levofloxacin, gentamicin and 100% susceptible to imipenem (Fig. 4).

DISCUSSION

The present study shows *Pseudomonas aeruginosa* with the highest frequency of occurrence of 48.6% followed by *Staphylococcus aureus* and *Escherichia coli* with a percentage occurrence of 23% and 14.9% respectively. *Klebsiella pneumoniae* was least occurring with 13.5% of the total bacteria isolates. Out of the seventy-four (74) isolates, fifty-seven (77%) were Gram-negative while seventeen (23%) were Gram-positive with *P. aeruginosa* being the most prevalent Gram-positive organism. The data regarding the above clinical isolates and their prevalence in wound infection is consistent with other studies reported from Nigeria and outside. [6, 10, 27-36] As shown in the results of this study, majority of the patients with wound infection were males with 64% incidence rate as opposed to 36% for females. The result obtained is also consistent with previous studies within and outside Nigeria. [14, 15, 37-39] It is likely due to the fact that males all over the world are much more involved in construction works, transportation, industry works, farming, fishing and lots of other physical activities that may damage an area of the body more than females. [1]

In the present study, twenty-six percent (26%) of the samples showed poly-microbial growth while fifty-four percent (54%) showed mono-microbial growth. This result is similar, to an earlier study in India (86-100%) and Pakistan (98%), where high percentage of mono-microbial growth was reported. [40-42] The poly-microbial growth showed a pattern of higher prevalence of co-infections involving *P. aeruginosa* and *S. aureus* (42%) followed by *P. aeruginosa*/*K. pneumoniae* (21.1%), *P. aeruginosa*/*E. coli* (21.1%), *P. aeruginosa*/*K. pneumoniae*/*S. aureus* (10.5%), and finally *P. aeruginosa*/*S. aureus*/*E. coli* (5.3%). The increased cases of *P. aeruginosa* and *S. aureus* occurring together among the poly-microbial infections are in line with an earlier study. [31-32] In acute soft tissue infections, polymicrobial

aerobic-anaerobic interactions are reported to play a major role in disease progression and severity. [3] Amongst the Gram-negative bacteria, *K. pneumoniae* showed the highest resistance rate, being resistant to over 90% of the antibiotics used against it, but susceptible to imipenem. *P. aeruginosa* showed susceptibility to four of the test antibiotics as opposed to *E. coli* which showed multiple drug resistance pattern to all the test drugs except imipenem. *S. aureus*, the only Gram-positive bacteria was found to be 50% susceptible and 50% resistant to the test antibiotics.

As showed in Fig. 2, susceptibility of *S. aureus* to vancomycin (65%) differed with previous studies in South Africa by Ferraz *et al*. [43] and Amod *et al*. [44] In those reports, *S. aureus* showed intermediate resistance to vancomycin possibly due to difference in environmental factors, drug misuse and genetic factors of the bacterium contributing to their sensitivity or resistance. The pattern of *S. aureus* resistance to erythromycin, oxacillin, trimethoprim obtained in this study was however similar to earlier an study by Fanelli *et al*. [45] comparing the susceptibility patterns between the patients who are using antibiotics and those who are not using antibiotics. The overall multiple drug resistance (that is, resistance to two and above antimicrobial classes) of the bacteria isolates in this study was >85% which was in agreement with previous study by Biadglegne *et al*. [14] Mulu *et al*. [46] and Bayram *et al*. [47] The susceptibility rate of *P. aeruginosa* varied compared with the sensitivity patterns to different anti-pseudomonal drugs reported worldwide. Previous studies conducted at different parts of the world showed *P. aeruginosa* was susceptible to ceftazidime as compared to the result obtained in this study. [48-49] In this study, *P. aeruginosa* is shown to be resistant against ceftazidime, but susceptible to gentamicin and ciprofloxacin as previously reported. [50-52] These variations in sensitivity patterns of *P. aeruginosa* may be due to the condition of the environment, genetic variations or the misuse of these antibiotics by patients. For *E. coli*, resistance to antimicrobials was high, with ceftazidime, aztreonam, amoxicillin-clavulanic acid at the top (100%), followed by polymixin B and erythromycin, 73% resistance to sulfamethoxazole-trimethoprim, 64% resistance to ciprofloxacin, levofloxacin, gentamicin and 100% susceptible to imipenem. The non-inhibition of *E. coli* may be as a result of the frequent use of these drugs by patients. Similar studies in Slovenia by Petkovsjek *et al*. [53] presented erythromycin as not being active against the test organism and in agreement with earlier studies carried out by Orrett and Shurland, [54] Bharathi *et al*. [55] Briscoe *et al*. [56] Kurutepe *et al*. [57] Iqbal *et al*. [58] For *K. pneumoniae*, the pattern of antibiotics activity presented in Fig. 3 seems to be consistent with earlier study conducted in Ethiopia by Biadglegne *et al.* [14] Mulu *et al*. [22] Endalafer *et al*. [59] Although, its resistance to ciprofloxacin in this study was higher (90%) than the reported resistance in the study done in Ethiopia (35.7%). The response of the different microorganism and their effect on wound has been widely reported. *S. aureus* and *P. aeruginosa* are associated the most with delayed healing; this might be because of their ability to produce potentially destructive virulence factors than others. [3, 8, 60]

Previous report shows that cooperation amongst microorganisms can play a role in the net sensitivity or resistant pattern of the organisms and/or the degree of infection. [3, 61] This could occur in many ways, some of which includes the depletion of available oxygen by aerobic organisms, the production of specific nutrient by one organism encouraging the growth of fastidious and potentially pathogenic organisms cohabiting together. [3] The anaerobes provide a competitive advantage amongst themselves because of their ability to impair host cell function. [3] Nevertheless, the efficacy of the host immune response in dealing with wound microflora is an important factor in wound healing and infection. [3] As earlier noted, microorganisms can as well contribute to the disruption of immune response thereby putting the patient at greater risk of infection. The likelihood of an infection in this instance can be addressed by looking at the host and the invading microorganism together. As the controversy regarding the sensitivity to available antimicrobial agent continues, there is a need to identify and develop new antimicrobials that are broadly effective, safe and have low propensity to induce resistance. Also, the need to control microbial populations that inhabits wound surface to reduce the chances of infection, minimize their spread as well as eliminating the chances for cross-infection. [3] Several authors have recommended wound dressing as an important control measure that is capable of physically preventing and/or reducing the transmission of pathogenic organisms. [3, 62-63]

The most common microorganisms associated with wound infections in patients at the University of Port Harcourt Teaching Hospital in Port Harcourt, Nigeria are *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae.* The bacteria isolates showed moderate susceptibility to gentamicin, ciprofloxacin and levofloxacin, high resistance to ceftazidime, erythromycin, sulphamethoxazole/ trimethoprim, cefepime, amoxicillin/clavulanic acid and aztreonam. Imipenem is thus observed to be an excellent antimicrobial drug *in vitro.* High resistance by microorganisms to test antimicrobial agents raises further concerns about the misuse of these agents. Hence, the need to reinforce the rational use of antimicrobial agents and encouraging the practice of aseptic techniques at all levels to reduce the emergence and spread of resistant pathogens.

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