

Antibiotic Susceptibility Profile of Gram-Negative Isolates from Wound Swabs.

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ABSTRACT

Objectives: This study was carried out to isolate and identify gram negative bacteria in wounds and ascertain their antimicrobial susceptibility pattern.

Methods: One hundred and twenty-five wound swabs were collected from patients using cotton tipped swab sticks, inoculated by standard bacteriological techniques and the isolates identified by biochemical tests. Antimicrobial susceptibility testing was done by the disc diffusion method.

Results: Sixty-four isolates were obtained (51.2% prevalence). These include *Pseudomonas aeruginosa* (50.7%), *E. coli* (38.1%), *Proteus vulgaris* (6.3%) and *Proteus mirabilis* (4.8%). Ninety five percent of the isolates were resistant to 3 or more antibiotics. The 64 isolates were made up of 34 resistance phenotypes.

Conclusion: There is high prevalence of multiple antibiotic resistant gram-negative isolates in wounds of the patients. It is important that the antimicrobial susceptibility pattern of wounds be determined before initiating antimicrobial therapy to avoid selection of multidrug resistant strains. Appropriate infection control measures are also necessary to curtail the spread.

Key words: Antimicrobial, Infection, Resistance, Susceptibility, Wound.

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Author's contributions:

This work was carried out and approved in collaboration between all authors. ECP, OK, OTV, designed the study, ECP sourced for funding, ECP, OK, OTV wrote the protocol, ECP contributed in literature search, ECP, OK did statistical analysis, ECP wrote the manuscript. ECP did the experiments; OK, ECP managed literature searches, ECP, OK, OTV did data & statistical analysis, ECP wrote the manuscript, and OK, OTV proofread the manuscript.

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1. INTRODUCTION

The intact skin is the first line of defense against invading microorganisms. It is an anatomical/physical barrier that prevents most infectious agents from gaining access into the body. A break in this local defense means that the protective function of the skin is compromised and results to injury (wound); the underlying connective tissue may or may not be affected (1). This external damage to the skin varies in degree ranging from the very minor ones such as abrasions, minor cuts, lacerations and puncture wounds to more serious ones such as bites, burns and surgical incisions. These wounds could be acute as those mentioned above or chronic which refers to wound that has failed to heal within three months (2).

Bacterial colonization and infection of wound sites lead to increased morbidity and mortality among patients with wounds. This follows complications such as delayed healing and wound breakdown as noted by Alexander (3), prolonged hospital stay and increased cost of healthcare (4). Wound infections account for most hospital acquired infections (5).

When the skin is injured the protective defense mechanism is impaired. This creates a conducive environment for bacteria, which multiplies and grows in number, a condition referred to as colonization (6, 7). Though resident flora (skin flora and those from the gastrointestinal flora and other parts of the body) usually colonize the wounds, environmental microorganism (external flora) commonly colonize wounds and some eventually result to infections. Siddiqui &

Bernstein(2) and Edwards & Harding (8) classified wound association with microorganisms as contamination, colonization, local infection or critical colonization (which may present as delayed healing) and finally as spreading invasive infection and septicemia (systemic infections which results when there is hematogenous spread of the infection throughout the body presenting with symptoms such as fever, chills and tachycardia).

Bacteria associated with acute and chronic wounds include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococci*, Beta-hemolytic streptococci, *Escherichia coli*, *Enterobacter species* and *Klebsiella pneumoniae* (9). Others include Coagulase-negative staphylococci, Pigmented gram-negative anaerobes (*Prevotella* and *Porphyromonas species*), Non-pigmented gram-negative anaerobes (primarily *Bacteroides*, *Prevotella*, and *Fusobacterium species*), *Peptostreptococcus species* and *Clostridium species* (10). The aetiologic agents of wound infections vary greatly according to regional and local conditions (4, 11, 12).

To identify the specific organisms associated with wound infections and to guide specific antimicrobial therapy, wound cultures are undertaken (10, 13). It is indicated for surgical and non-surgical wounds, whether acute or chronic, and for hospital or local surveillances to monitor drug-resistant microorganisms (14)

Bacterium that exhibits simultaneous resistance to at least one antimicrobial drug in three or more different chemical classes is termed multidrug-resistant (MDR) (15). Treating wounds infected with such multidrug resistant microorganisms are a great challenge to clinicians and Microbiologists. Several studies have reported the aetiology and antimicrobial profile of wounds across Africa (16) and Nigeria (4, 8, 17, 18), but not much is reported from any of the tertiary health facilities in Owerri, South East Nigeria. The aim of this study was therefore to isolate and identify gram negative bacteria in different kinds of wounds in Owerri, Imo State, and ascertain their antimicrobial susceptibility pattern.

2. MATERIALS AND METHODS

Source of samples for the study

Wound swabs were collected from Federal Medical Centre, Owerri, Imo State, South East Nigeria.

Ethical approval was obtained from the Ethical Committee of the Federal Medical Centre, Owerri.

Samples:

One hundred and twenty five wound swab specimens were collected from both in-patients (n = 45) and out-patients (n = 80), using the appropriate cotton tipped swab sticks.

Culture

The wound swab samples were inoculated by standard bacteriological techniques as described by Cheesbrough (19).

The isolates were identified by Morphological and the following standard biochemical tests: Indole test, Methyl red test, Voges –Proskauer test, Citrate utilization test, Urease test, Sugar fermentation tests and Gelatin liquefaction test (20).

Antimicrobial susceptibility testing

Kirby Bauer's disc diffusion method was employed for antibiotic susceptibility testing of the organism (21)

Antibiotic discs used include: Cefotaxime (30µg), Ceftazidime (30µg), Levofloxacin (5µg), Imipenem (10µg), Ciprofloxacin (10µg), Ampicillin (10µg), Cefpodoxin (30µg), Aztreonam (30µg), Ceftriaxone (30µg), Gentamicin (10µg), Amoxicillin/clavulanate (20/10 µg). All antibiotic disks were obtained from Oxoid (England).

A suspension of the isolate was made on sterile water and the turbidity matched visually with 0.5 MacFarland standard. The suspension was spread on the surface of Mueller Hinton agar using sterile swab stick and the discs applied and incubated for 24 hrs. The diameter of zone of inhibition was measured and results recorded as sensitive, resistant or intermediate.

Multi Antibiotic Resistance Index (MAR) for the isolates were calculated using Krumperman (22) formula: a/b where a = number of antibiotics to which each individual isolate was resistant and b = number of antibiotics to which the isolate was exposed.

3. RESULTS

A total of 64 isolates of gram negative bacilli were obtained from 125 wound swab specimens (51.2%). These include *Pseudomonas aeruginosa* 32 (50.0%), *E. coli* 25 (39.1%), *Proteus vulgaris* 4 (6.3%) and *Proteus mirabilis* 3(4.7%).

Sixty out of the 64, isolates (93.8%) were resistant to 3 or more of all antibiotics used.

About 40.6% of the *Pseudomonas aeruginosa* isolates were resistant to gentamicin, 53.1% to ciprofloxacin, 59.4% to cefotaxime and ceftazidime while 87.5%, 96.9% and 100% were resistant to amoxicillin/clavulanate, ampicillin and aztreonam respectively. Only 12.5% were resistant to imipenem (figure 1).

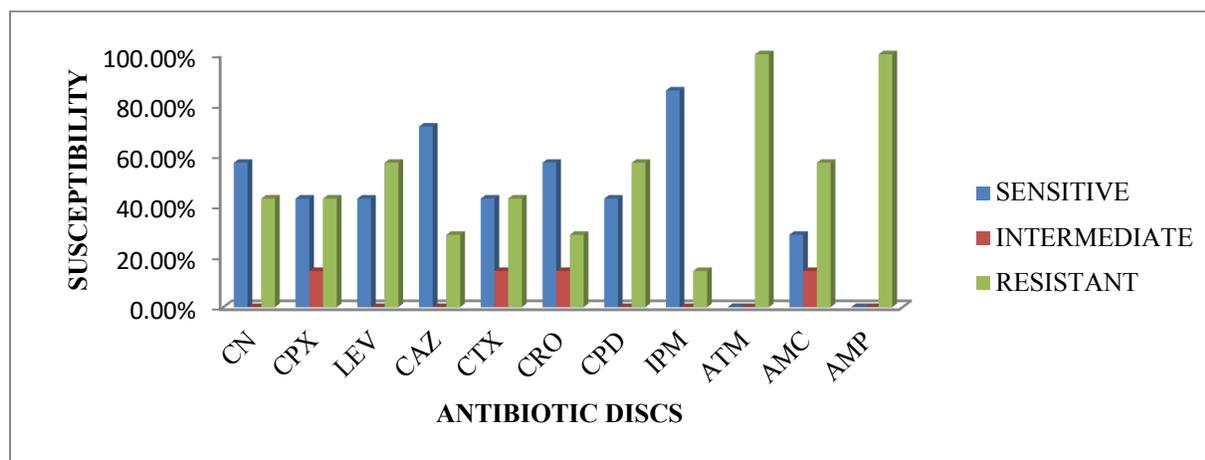


Figure 1: Susceptibility pattern of *Pseudomonas aeruginosa*:

Key: CN= GENTAMICIN, CPX= Ciprofloxacin, LEV= Levofloxacin, CAZ= Ceftazidime, CTX= Cefotaxime, CRO= Ceftriaxone, CPD= Cefpodoxime, IPM= Imipenem, ATM= Aztreonam, AMC= Amoxicillin-Clavulanate, AMP= Ampicillin

Out of the 25 *E. coli* isolates, 23(92.0%) were resistant to three or more antibiotics, nearly all (95.8%) were susceptible to imipenem. The

mean resistance to the 3rd generation cephalosporins was 54.1% while ampicillin and aztreonam have resistance of 87.5% and 79.2% respectively (Figure 2)

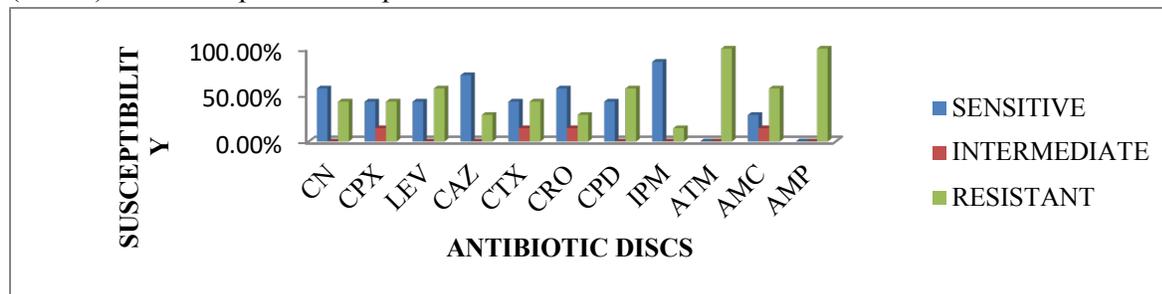


Figure 2: Susceptibility pattern of *E. coli* : KeyCN= GENTAMICIN, CPX= Ciprofloxacin, LEV= Levofloxacin, CAZ= Ceftazidime, CTX= Cefotaxime, CRO= Ceftriaxone, CPD= Cefpodoxime, IPM= Imipenem, ATM= Aztreonam, AMC= Amoxicillin-Clavulanate, AMP= Ampicillin.

As for the *Proteus species*, all (100%) were resistant to ampicillin and aztreonam

respectively, while only 14.3% were resistant to Imipenem (figure 3).

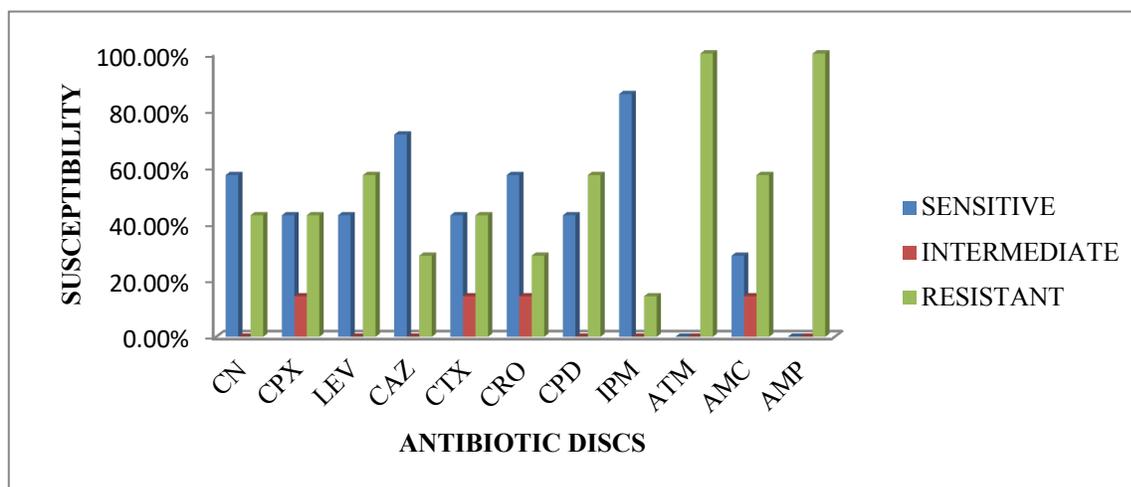


FIGURE 3: Susceptibility pattern of *Proteus species*

Key: CN= GENTAMICIN, CPX= Ciprofloxacin, LEV= Levofloxacin, CAZ= Ceftazidime, CTX= Cefotaxime, CRO= Ceftriaxone, CPD= Cefpodoxime, IPM= Imipenem, ATM= Aztreonam, AMC= Amoxicillin-Clavulanate, AMP= Ampicillin.

The 64 isolates were made up of 34 resistance phenotypes. The most prevalent resistant phenotype was ATM-AMC-AMP (with 10 isolates) and CN-CPX-LEV-CAZ-CTX-CRO-CPD-ATM-AMC-AMP (with 11 isolates) respectively. Sixty four percent of all the isolates had multiple antibiotic resistance (resistant to at least one from each of the 3 classes of antibiotics used).

Pseudomonas aeruginosa had 19 resistant phenotypes with ATM-AMC-AMP as the most

prevalent while *E. coli* had 16 resistant phenotypes with CN-CPX-LEV-CAZ-CTX-CRO-CPD-ATM-AMC-AMP as the most prevalent.

Fifteen out of 25 *E. coli* isolates (60%) were resistant to at least one antibiotic from each of the three classes of antibiotics used (Aminoglycosides, Quinolones and Beta-lactams) (Table 1).

Table 1: Multiple Antibiotic Resistant Index of all Isolates

MAR Index	<i>Pseudomonas aeruginosa</i> (n=22)	<i>E. coli</i> (n = 15)	<i>Proteus spp.</i> (n =4)
0.45	1	3	-
0.55	1	3	-
0.64	2	-	1
0.73	4	2	2
0.82	5	1	-
0.91	6	6	-
1.00	3	-	1

Similarly, 68.75% of *Pseudomonas aeruginosa* isolates were Multiple Antibiotic Resistant with an MAR Index ranging from 0.45 (resistant to 5 antibiotics, at least 1 from each of the 3 classes) to 1.0 (resistant to all 11 antibiotics). *Proteus species* had MAR index of 0.64 (Resistant to 7 antibiotics) to 1.00 (resistant to all 11 antibiotics)

4. DISCUSSION

The prevalence rate of gram-negative wound infection in this study was 51.2%. This prevalence differs a bit from 57% reported by Mohammed *et al* (16) in Ethiopia. It is less than the 85.05% reported by Pondei *et al* (17) at Okolobiri, Nigeria. These findings underscore the severity of gram-negative bacteria in wound infections.

Pseudomonas aeruginosa was the most prevalent isolate (50.0%) followed by *Escherichia coli* (39.1%), similar to the report of Pondei *et al* (17).

About 64% of all the isolates had multiple antibiotic resistance (resistant to at least one from each of the 3 classes of antibiotics used). This poses a challenge in the choice of antibiotics for treatment of such wounds.

Pseudomonas aeruginosa had 100% resistance to the monobactam -Aztreonam, 96.9% resistance to Ampicillin and 87.5% resistance to Amoxicillin/clavulanate. The resistance to the fluoroquinolone ciprofloxacin and the third generation cephalosporins were above 50% respectively. This limits the choice of antibiotics to the carbapenem – imipenem with the least resistance of 12.5%. The resistance pattern of *E. coli* was similar to that of *Pseudomonas* but with greater susceptibility to imipenem (95.8%).

The high Multiple Antibiotic Resistance Index discovered in this study is worrisome because

most of the antibiotics used in the study are the most commonly prescribed (23, 24, 25). Six *Pseudomonas* isolates and 6 *E. coli* isolates have very high MAR Index of 0.91 (that is, resistant to 10 out of 11 antibiotics) while 3 *Pseudomonas* and 1 *Proteus* isolates have infinite MAR Index of 1.0 (resistant to all 11 antibiotics used). The reason for the resistance could be attributed to frequent drug abuse in the society. Several inappropriate antibiotics uses such as self-medication, over use of antibiotics and under dosage/noncompliance with treatment regimen are practiced in this part of the world. For instance, there is high tendency for the patient to stop administration of prescribed antibiotics half way once symptoms seem to have resolved. These are factors already known to facilitate development of resistance to antibiotics (18, 26, 27). Another probable reason for development of multiple antibiotic resistance is the application of empirical approach in initial treatment of wound infections. Proper identification of the etiologic agents of wound infections and determination of the antibiogram will go a long way to tackle these infections with greater accuracy, prevent development of multiple antibiotic resistance and reduce the morbidity and mortality associated with wound infections as well as save cost for the patients.

5. CONCLUSION AND RECOMMENDATION

There is high prevalence of multiple antibiotic resistant gram-negative isolates in these wounds. It is important that the antimicrobial susceptibility pattern of wounds be determined before initiating antimicrobial therapy to avoid selection of multiple antibiotic resistant strains. Appropriate infection control measures are also necessary to curtail the spread of multiple antibiotic resistant strains.

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