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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF PATHOGENS ASSOCIATED WITH PRESSURE INJURY IN IMMOBILIZED PATIENTS AT A TERTIARY HOSPITAL IN PORT HARCOURT, NIGERIA.

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ABSTRACT

Aims: This study was done to determine the prevalent microorganisms associated with pressure injuries of immobilized patients and their antibiotic susceptibility patterns. **Methods**: This was a cross sectional prospective study done at University of Port Harcourt Teaching Hospital (UPTH) in Port Harcourt, Nigeria. Approval was obtained from the Research and Ethics Committee of the UPTH. Samples were collected from 12 patients with pressure injuries at different wards of the UPTH and analyzed using standard microbiological methods. Antibiotic susceptibility testing was done using Bauer-Kirby disk diffusion method. **Results**: A total of 35 bacterial and 12 fungal isolates were obtained. The bacterial isolates in order of decreasing frequency of occurrence were *Pseudomonas aeruginosa,* 12 (34.3 %), *Staphylococcus aureus,8* (22.8 %), *Klebsiella pneumoniae,* 7 (20 %), *Escherichia coli* 5 (14.3 %) and Coagulase *negative Staphylococci* 3 (8.6 %). The fungal isolates were *Aspergillus fumigatus, Aspergillus niger, Penicillium notatum* and *Penicillium chrysogenum*. Although *Escherichia coli* was 100% and 75% susceptible to gentamicin and levofloxacin respectively, a high level of multidrug resistance was seen among the isolates. *Klebsiella pneumoniae* was 100% resistant to clindamycin and erythromycin, and resistant to levofloxacin, tetracycline and ceftriaxone to varying degrees. A striking finding of this study was the development of 100 % resistance to ceftriaxone and cefoxitin by all *Staphylococcus aureus* and Coagulase negative *Staphylococci* isolated. **Conclusion:** Improved nursing care and stricter infection prevention and control mechanisms to combat transmission of multidrug resistant organisms in the hospital is urgently recommended.

KEYWORDS: pressure injuries, immobilization, multidrug resistance.

INTRODUCTION

Immobilization refers to the state in which an individual has a limitation of independent, purposeful physical movement of the body or of one or more lower extremities.[1] The International Medical Prevention Registry on Venous Thromboembolism study also defined immobilization as hospitalization or bed rest > 7 days.[2] Prolonged immobility has multiple effects on the major systems of the body and can result in a negative physiologic response in hospitalized patients on bed rest. Immobility in health care settings is very critical and can lead to several other complications which may be detrimental to the patient's health.[3]

A pressure injury has been defined as an area of unrelieved pressure over a defined area, usually over a bony prominence, resulting in ischemia, cell death and tissue necrosis.[4-6] Pressure ulcers or injuries develop rather quickly, and if left untreated, can progress into more serious and difficult to treat stages.[7-9]

Pressure injuries are also highly infectious and these infections can be the beginning of other serious complications, some of which can be life threatening.[10] Body tissues differ in their ability to tolerate pressure, with muscle being more sensitive to pressure damage than skin. Pressure ulcers continue to be a common health problem, particularly among immobilized patients. The pain and discomfort of pressure ulcer delays rehabilitation, prolongs illness, and timing of discharge, and also contribute to disability and death. These dramatically raise health care costs as a result of the need for supplies and nursing hours. The problem exists within the entire health framework, including hospitals, clinics, long-term care facilities and private homes.[11]

Pressure injuries are staged based on depth and damage to the skin ranging from non-blanching erythema of intact skin to full-scale tissue destruction.[12] Despite the great successes in medicine, burden of pressure injuries remains significantly huge and is ranked as the third costing disease after cancer and cardiovascular diseases.[13] The prevalence of microorganisms in

pressure injuries is usually polymicrobial and complex and these injuries if not managed properly can be a source of infection with resistant microorganisms in hospitalized patients.[14-16]

The presence of microorganisms in pressure ulcers plays a significant role in the normal process of healing.[17] The prevalence and susceptibility of various bacterial as well as fungal species vary between hospitals, wards and among various patient populations in the same hospital. Antimicrobial resistance has long been established to be on the rise globally and physicians have the responsibility of making good and effective clinical judgements based on prevalence and susceptibility patterns of commonly implicated pathogens in pressure injuries. Conducting periodic studies aimed at identifying, combating and counteracting new and emerging trends of resistance has thus become imperative.

This present study was therefore undertaken to determine the prevalence of pathogenic microbes commonly implicated in pressure injuries in immobilized patients at the UPTH and their antibiotic susceptibility patterns and to compare the rate of isolation of potential pathogens from pressure injuries in different body sites and different clinical wards with a view to directing appropriate antibiotic stewardship.

MATERIALS AND METHODS

Materials

Culture Media

These included nutrient agar, nutrient broth, mannitol salt agar, MacConkey agar, cetrimide agar, Mueller Hinton agar, Sabouraud's dextrose agar (SDA) and peptone water all made by Titan Biotech limited, India.

All media were prepared according to manufacturers' specifications and sterilized by autoclaving at 121^{o}C for 15 minutes.

REAGENTS

Indole (Kovac's) reagent, Catalase reagent, Sterile Peptone water, Lugol's iodine, Crystal violet, 95% alcohol, 1% safranin red, 95% alcohol, Oxidase reagent, and Purit ® were used in the study.

Ethical Approval

This study was approved by the Research and Ethics Committee of the UPTH (UPTH/ADM/90/S.II/VOL.XI/834) following submission of a research proposal and application for approval. Samples were taken from patients after they or their legal guardians had given their informed voluntary consent to be a part of the study. Standard protocols were followed to ensure confidentiality of patient information.

Inclusion / Exclusion Criteria

Participants who met the immobilization criterion who had pressure injury and gave their informed voluntary consent were enrolled into the study while those who did not consent to participate in the study were not included.

Sociodemographic Data Collection

A standard structured questionnaire was used to collect information concerning the 12 patients with pressure ulcers or injuries from whom samples were collected. The questionnaires provided basic information such as antibiotics in use, frequency of dressing of pressure injury and site of pressure injury among others.

Data Analysis

Data generated were analyzed using version 21.0 of the Statistical Package for Social Sciences (SPSS). A probability of ≤ 0.05 was considered to be significant.

SAMPLE COLLECTION

Twelve samples of wound swab were collected from pressure injuries of immobilized patients, both geriatrics and pediatrics, in Orthopedic, Surgical and Medical wards of the hospital using sterile swab stick. Two swabs per patient were collected, one for bacterial culture and the other for fungal culture. The swab sticks were dipped in normal saline before swabbing was done. The swabbing was done by slowly rotating the swab over the wound. Each swab was put in a different sterile specimen container and properly labelled with appropriate codes and dates of collection. The samples were taken to the Pharmaceutical Microbiology and Biotechnology laboratory at the University of Port Harcourt for immediate microbiological analysis.

Inoculation method

The swab stick tips were cut off aseptically using flamed and cooled pair of scissors into bijou bottles containing nutrient broth. The bijou bottles containing nutrient broth were incubated at 37^{o}C for 24 hours. The swab stick for fungi was used to make a smear on the Sabouraud's dextrose agar (SDA) and streaked across the agar surface. The agar was incubated at ambient temperature for 7days.

ISOLATION AND CHARACTERISATION OF MICRO ORGANISMS

The isolation of the organism(s) was done using the streak plate method. A loopful of the nutrient broth culture was aseptically collected and streaked across the agar surface of the cetrimide nutrient agar, mannitol salt agar, MacConkey agar and nutrient agar respectively. The plates were incubated in an 'upside-down' position at 37 ^{o} C for 24 hours and observed for growth.

IDENTIFICATION OF ISOLATES

Gram staining and Biochemical Tests

Preliminary identification of the isolates was done using morphological characteristics of colonies (shape, color, size, edge and number of colonies). Gram staining test was done to preliminarily classify microorganisms into either Gram-negative or Gram-positive organisms. Biochemical tests which are based on the ability of some

bacteria to utilize certain enzymes to degrade carbohydrates, proteins or amino acids were done to identify and characterize unknown bacteria. All biochemical tests: indole, catalase, citrate utilization test, oxidase, coagulase, triple sugar iron test and urease tests were carried out using standard methods.[18]

IDENTIFICATION OF FUNGAL ISOLATES

Fungal cultures were identified based on colonial morphological characteristics.

ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

The antibiotics used in the susceptibility testing were Amoxicillin/Clavulanate (30µg), Cefoxitin(30µg), Ceftriaxone (30µg), Clindamycin(2µg), Tetracycline (30µg), Erythromycin (15µg), Gentamicin (30µg), and Levofloxacin (5µg). Fluconazole (1 ug) was used for the fungal isolates.

Antibiotic susceptibility test was done using the Bauer-Kirby disk diffusion[19] method. Single antibiotic discs were placed on the agar aseptically and incubated for 24 hours at 37 ^{o} C. The same procedure was carried out for fungal isolates but Sabouraud's dextrose agar (SDA) was used instead, after which, the plates were placed on the shelf for three to seven days. The inhibition zone diameters were measured and interpreted as resistant, intermediate or susceptible according to Clinical Laboratory Standards Institute (CLSI) guidelines for antimicrobial susceptibility testing.[20]

RESULTS AND DISCUSSION

Table 1: Socio-demographic characteristics of Study Participants.

Age Distribution of Participants	
Age Groups in Years	Number of Participants
36 – 45	*2(16.6%)*
46-55	2(16.6%)
56-65	4(33.3%)
66-75	3(25.0 %)
76- 85	1(8.3%)
Distribution of Participants by gender	
Male	5 (41.7 %)
Female	7 (58.3 %)
Educational Qualification	
Primary Education	0 %
Secondary Education	3 (25 %)
Tertiary Education	9 (36.7%)
Marital Status	
Single	4(33.3 %)
Married	5(41.7 %)
Widowed	3 (15%)
Divorced	0 %
Occupational Distribution	
Self Employed	4 (33.3 %)
Civil Servants	8(66.7 %)
Religion	
Christianity	12(100%)
Muslim	0 %

Table 2: Clinical Details of the Pressure Ulcers.

S/No	Period of immobilization (Days)	Site of Pressure Ulcer (PU)	Duration of Pressure Ulcer (Days)	Pressure Ulcer Dressing?	Frequency of Dressing PU	Antibiotic ?	Name of Antibiotic	Duration of Antibiotic
1	90	Sacrum	180	Yes	Daily	Yes	Cefuroxime	3months
2	160	Sacrum	120	Yes	Every 2days	Yes	Levofloxacin	5months
3	45	Sacrum	≥60	Yes	Daily	Yes	Ceftriaxone	1 week
4	180	Sacrum	90	Yes	Daily	Yes	Ceftazidime	5months
5	180	Ear	≥30	Yes	Daily	Yes	Ceftazidime	5months
6	90	Sacrum	30	Yes	Every 2Days	Yes	Metronidazole /cefuroxime	1month
7	50	Sacrum	30	Yes	Every 2Days	Yes	Levofloxacin	1month
8	180	Sacrum	120	Yes	Every 2days	Yes	Levofloxacin	5months
9	60	Sacrum	30	Yes	Every 4Days	Yes	Levofloxacin	2weeks

10	50	Heel	30	Yes	Every 2days	Yes	Levofloxacin	1 month
11	50	Greater Trochanter	30	Yes	Every 2days	No	Nil	Nil
12	30	Sacrum	30	no	No dressing	yes	Amoxicillin/Clavulanic Acid	11 days

Prevalence of Bacterial species in Pressure ulcers

The number and percentage occurrence of the bacteria isolated are presented in Table 3 below. *Pseudomonas aeruginosa* had the highest frequency of occurrence followed by *Staphylococcus aureus* and *Klebsiella pneumoniae* respectively.

Table 3: Number and Percentage occurrence of bacteria isolated from the 12 samples collected

S/N	Bacterium	No of Occurrence	Percentage Occurrence (%)
1	*Escherichia coli*	5	14.3%
2	*Staphylococcus aureus*	8	22.9%
3	Coagulase Negative *Staphylococcus*	3	8.6%
4	*Pseudomonas aeruginosa*	12	34.3%
5	*Klebsiella pneumoniae*	7	20.0%
	Total	35	100%

Table 4: Number of Bacterial Isolates and the Wards of Isolation.

Ward	Type and number of bacteria isolated from each ward				
	Pseudomonas aeruginosa	***Klebsiella pneumoniae***	***Staphylococcus aureus***	***Escherichia coli***	**Coagulase negative *Staphylococcus***
Male orthopedic ward	4	3	4	1	
Female orthopedic ward	2	1	1	1	
Female medical ward I	3	1	1	2	2
Female surgical ward	2	1	1	1	1
Male accidents & emergency ward	1	1	1		
Total	12	7	8	5	3

POLY- MICROBIAL COLONIZATION

The study revealed a high prevalence of polymicrobial colonization. Over 90% of the patients sampled were colonized by ≥ 3 multidrug resistant pathogens as shown in Table 5 below making the management of such patients difficult for the clinician.

Table 5: Pattern of Poly- microbial Colonization in the Sampled Population.

S/N	Organisms occurring together	Number	Percentage Occurrence (%)
1	*Pseudomonas aeruginosa/Klebsiella pneumoniae*	1	8.3%
2	*Pseudomonas aeruginosa/Staphylococcus aureus/Escherichia coli*	3	25%
3	*Pseudomonas aeruginosa/Staphylococcus aureus/Klebsiella pneumoniae*	5	41.7%
4	*Pseudomonas aeruginosa*/Coagulase Negative *staphylococcus/Klebsiella pneumoniae*	1	8.3%
5	*Pseudomonas aeruginosa*/ Coagulase Negative *staphylococcus /Escherichia coli*	2	16.7%
	Total	12	100%

Prevalence of Fungal species in Pressure ulcers

A total of twelve fungal organisms were isolated from the samples collected from the different patients seen at the hospital. The number and distribution of the fungal isolates were as presented in Table 6 below. *Aspergillus fumigatus* accounted for 50% of the isolates.

Table 6: Number and Percentage occurrence of fungi isolated from the 12 samples collected.

S/N	Micro organisms	No of occurrence	Percentage occurrence (%)
1	*Aspergillus fumigatus*	6	50
2	*Aspergillus niger*	1	8.33
3	*Penicillium notatum*	1	8.33
4	*Penicillium chrysogenum*	4	33.33
	Total	12	100

Colonial morphology of Fungal types isolated

Colonial morphology varies for different classes of fungi and colonial morphology alone is not a confirmatory test for identification of fungi. They were however used as a preliminary test to differentiate one fungus from another and the results are shown in Table 7 below.

Table 7: Colonial morphology of fungal isolates from Pressure ulcer

S/no	Colonial morphology	Suspected Fungal isolates
1	Green, dense velvet mycelium	*Aspergillus fumigatus*
2	Clusters of dark colonies	*Aspergillus niger*
3	Light green powdery circles	*Penicillium notatum*
4	White and powdery on the surface	*Penicillium chrysogenum*

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL AND FUNGAL ISOLATES

All isolates showed varied responses to the antimicrobial agents used in the tests. The figures below show the susceptibility pattern of organisms commonly isolated from pressure injuries at the University of Port Harcourt Teaching Hospital.

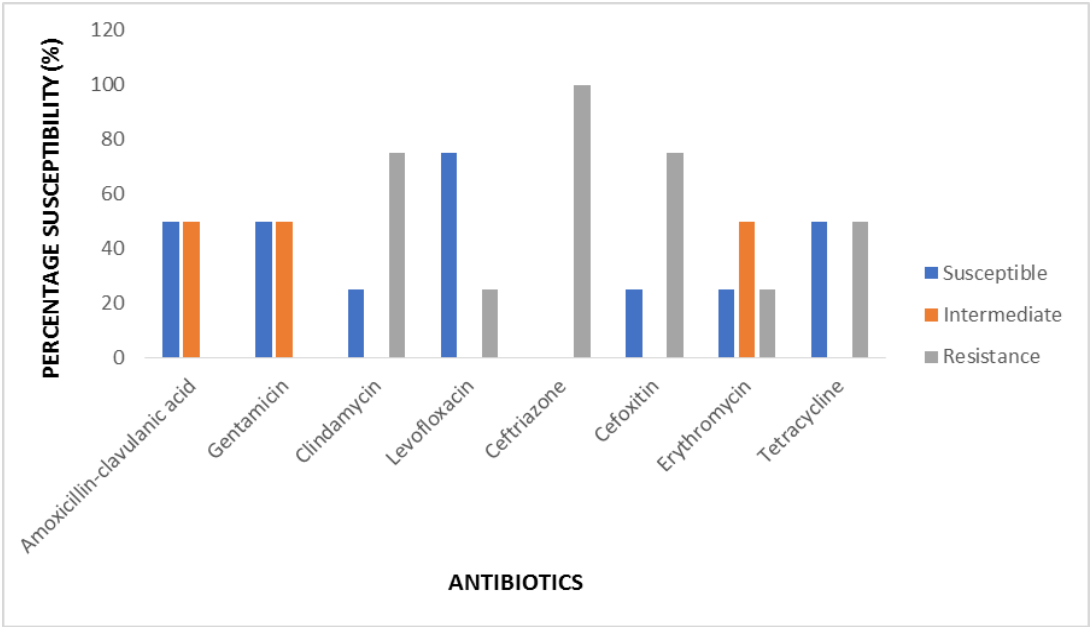

Fig. 1a: Susceptibility pattern of *Staphylococcus aureus* in Male Orthopedic Ward.

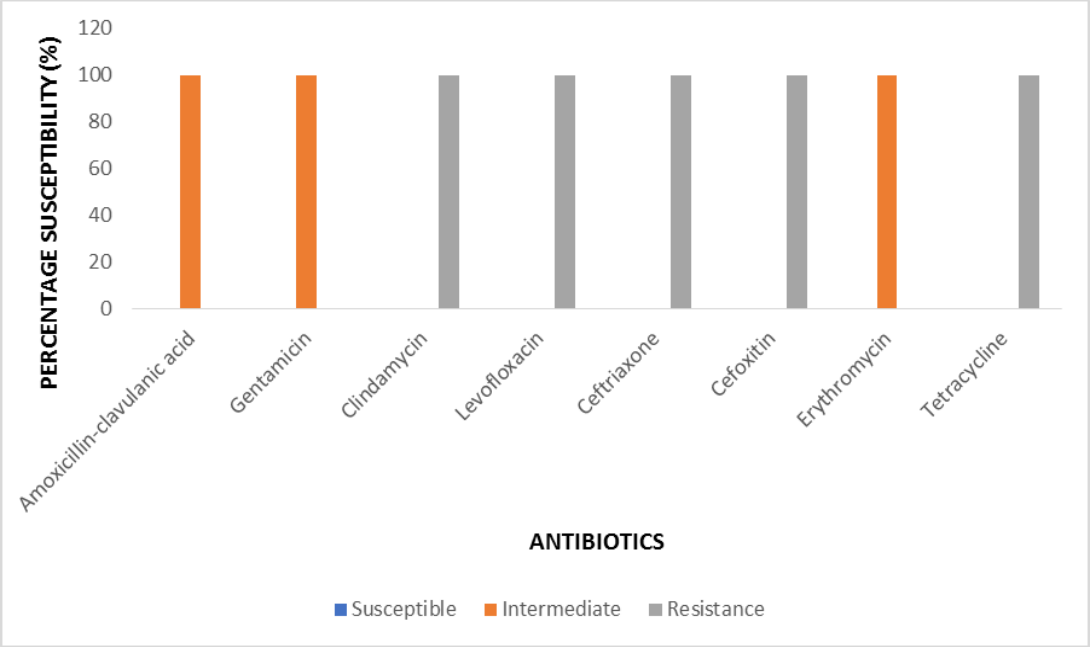

Fig.1b: Susceptibility Pattern of *Staphylococcus aureus* in Female Orthopedic Ward.

Fig. 2a below shows coagulase negative Staphylococci having almost 100% susceptibility to levofloxacin but being 100 % resistant to ceftriaxone, cefoxitin and erythromycin.

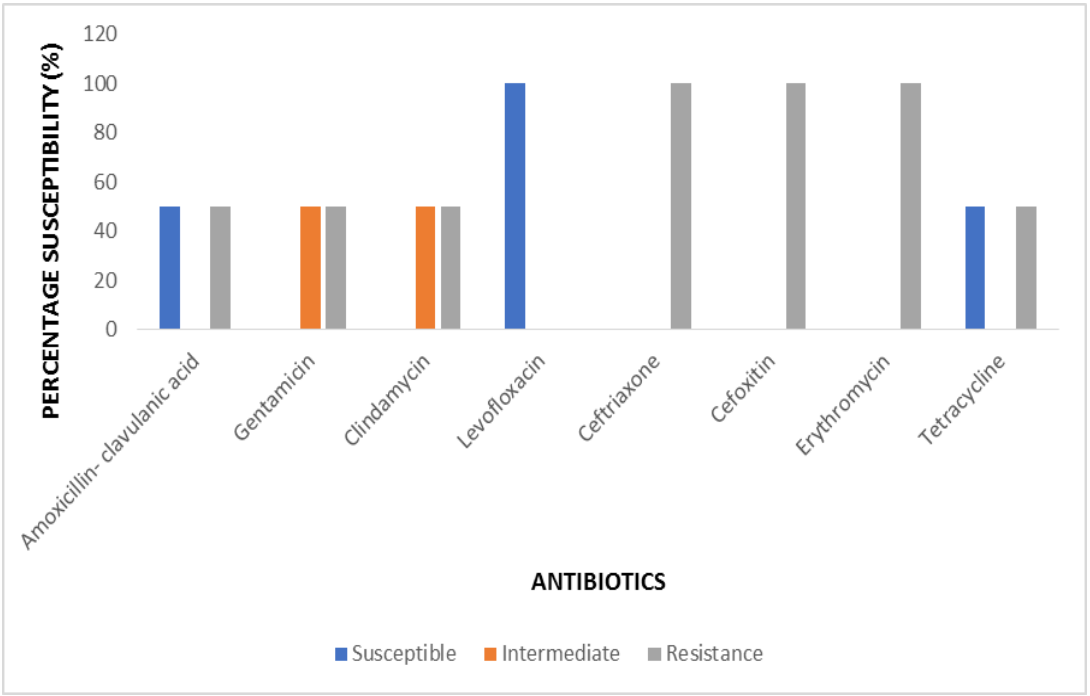

Fig. 2a: Susceptibility pattern of Coagulase negative *Staphylococci* isolated from Female Medical Ward 1.

In Fig.2b below Coagulase negative *Staphylococci* isolated from Female Surgical Ward exhibited 100% susceptibility to amoxicillin-clavulanic acid, levofloxacin and erythromycin while remaining 100% resistant to ceftriaxone and cefoxitin.

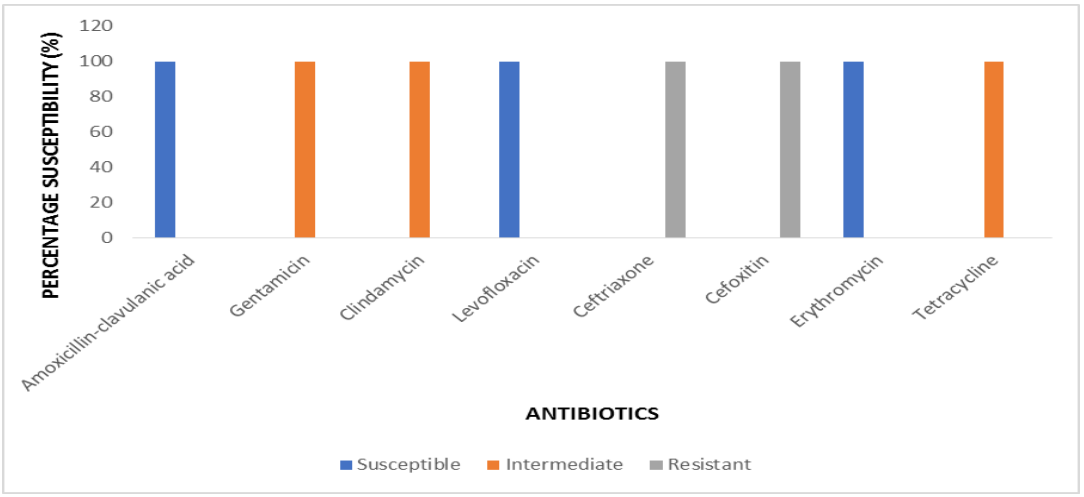

Fig.2b: Susceptibility pattern of Coagulase negative *Staphylococci* isolated from in Female Surgical Ward.

In Fig.3a below, another interesting result was seen where *Pseudomonas aeruginosa* isolated from Female Orthopedic Ward was 100 % resistant to levofloxacin, a traditional antipseudomonal drug, but 100% susceptible to cefoxitin and moderately susceptible to ceftriaxone, amoxicillin -clavulanic acid, clindamycin and tetracycline.

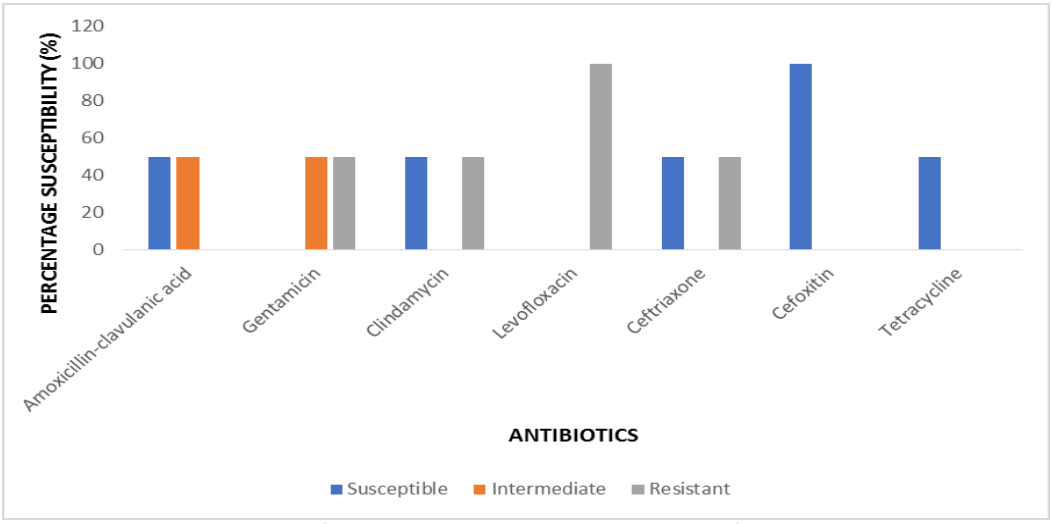

Fig.3a: Susceptibility pattern of *Pseudomonas aeruginosa* isolated from Female Orthopedic Ward.

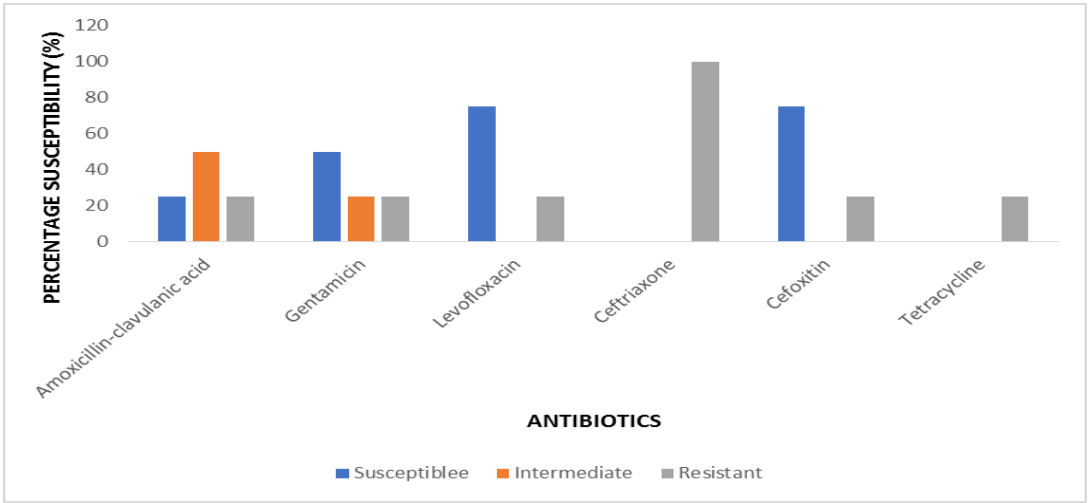

Fig.3b: Susceptibility pattern of *Pseudomonas aeruginosa* in Male Orthopedic Ward.

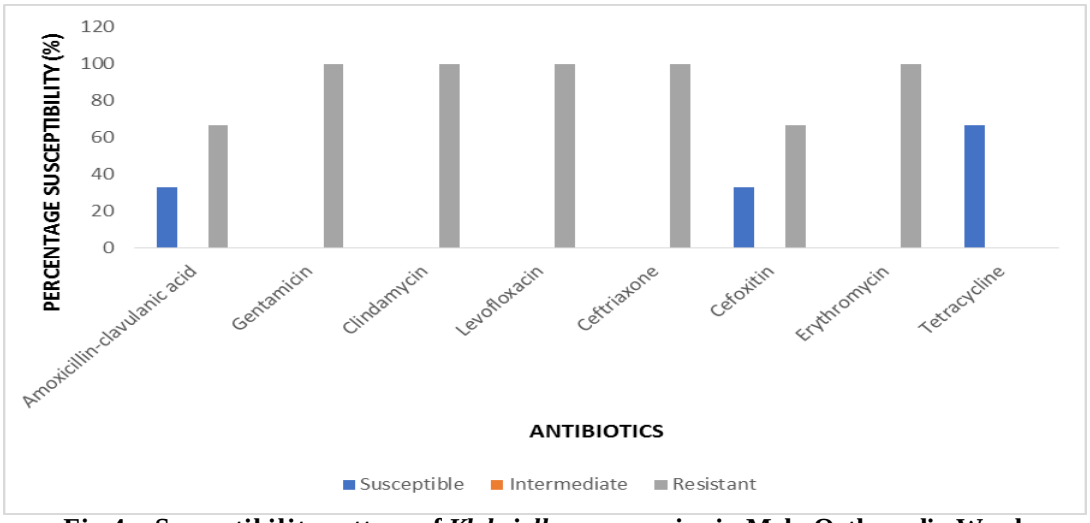

Fig.4a: Susceptibility pattern of *Klebsiella pneumoniae* in Male Orthopedic Ward.

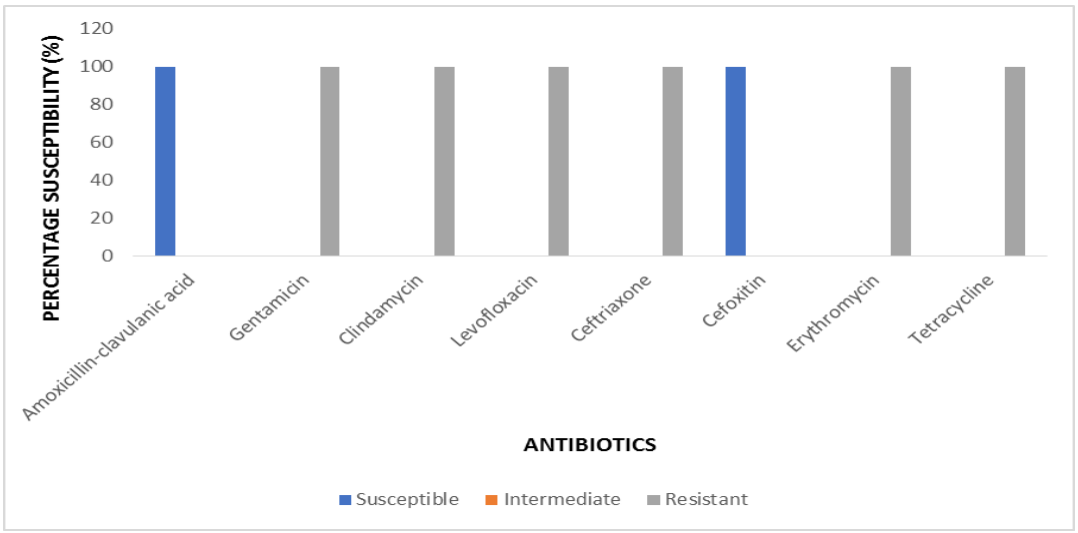

Fig.4b: Susceptibility pattern of *Klebsiella pneumoniae* in Female Orthopedic Ward.

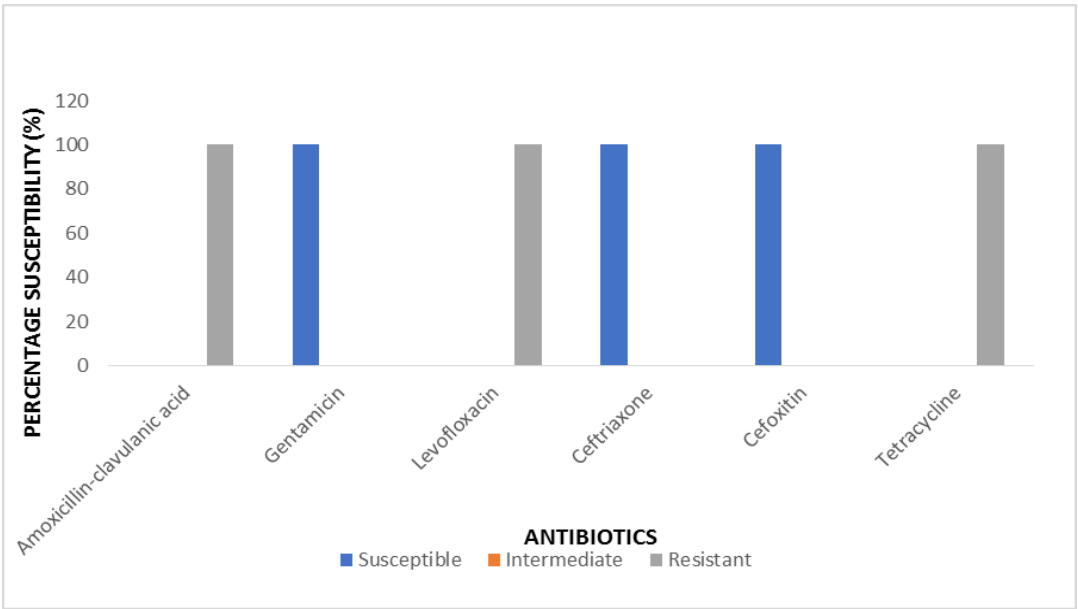

Fig. 5a: Susceptibility pattern of *Escherichia coli* in Male Orthopedic Ward.

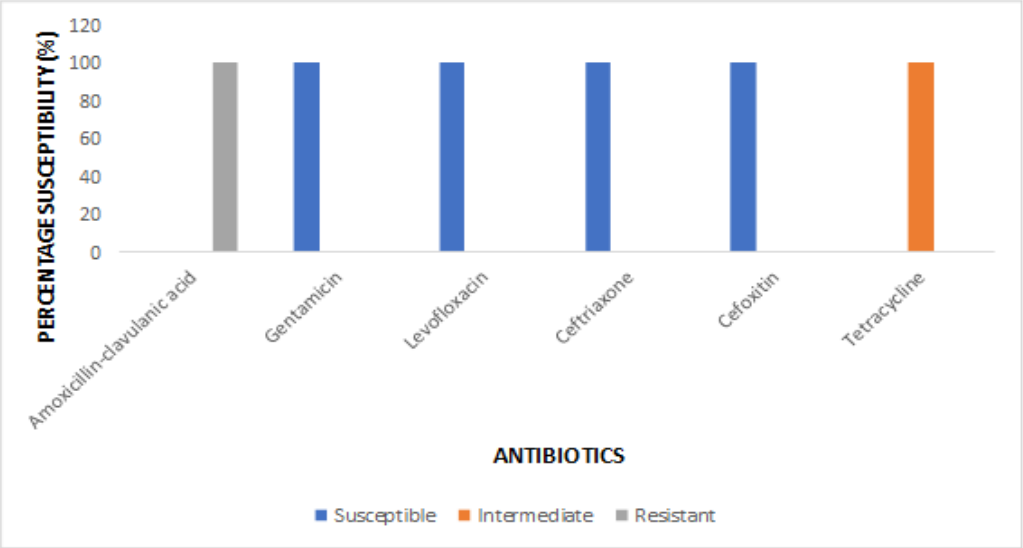

Fig. 5b: Susceptibility pattern of *Escherichia coli* isolated from Female Orthopedic Ward.

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF FUNGI ISOLATES.

The 12 fungal isolates showed 100% resistance to fluconazole across the various wards. The result for *Aspergillus fumigatus* and *Penicillium chrysogenum* is shown in Figs 6a and 6b below.

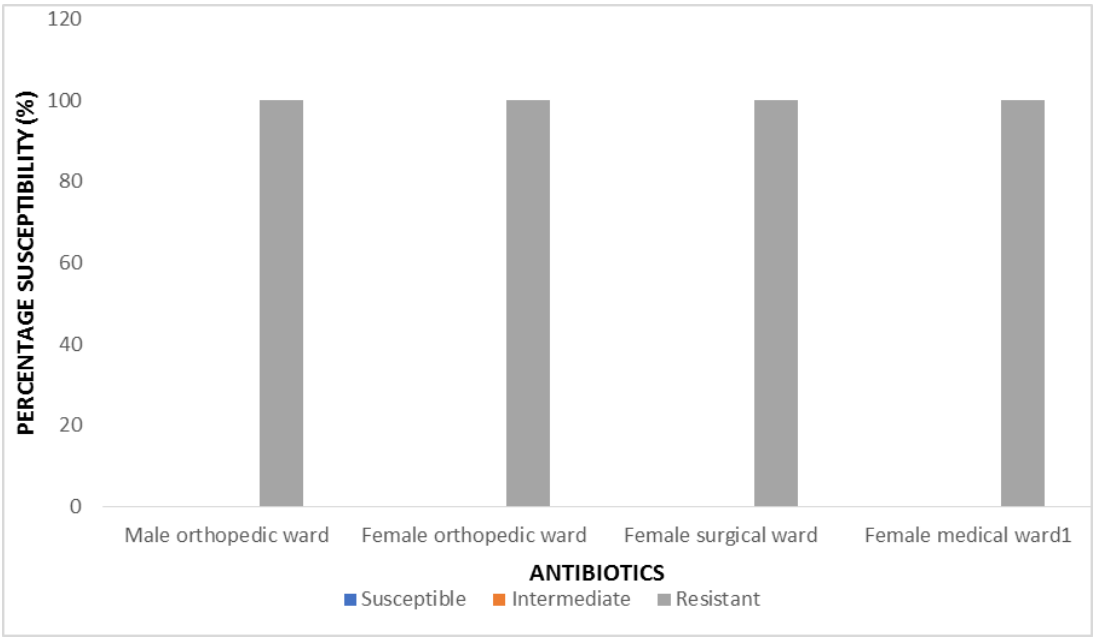

Fig.6a: Susceptibility pattern of *Aspergillus fumigatus* to fluconazole across different wards.

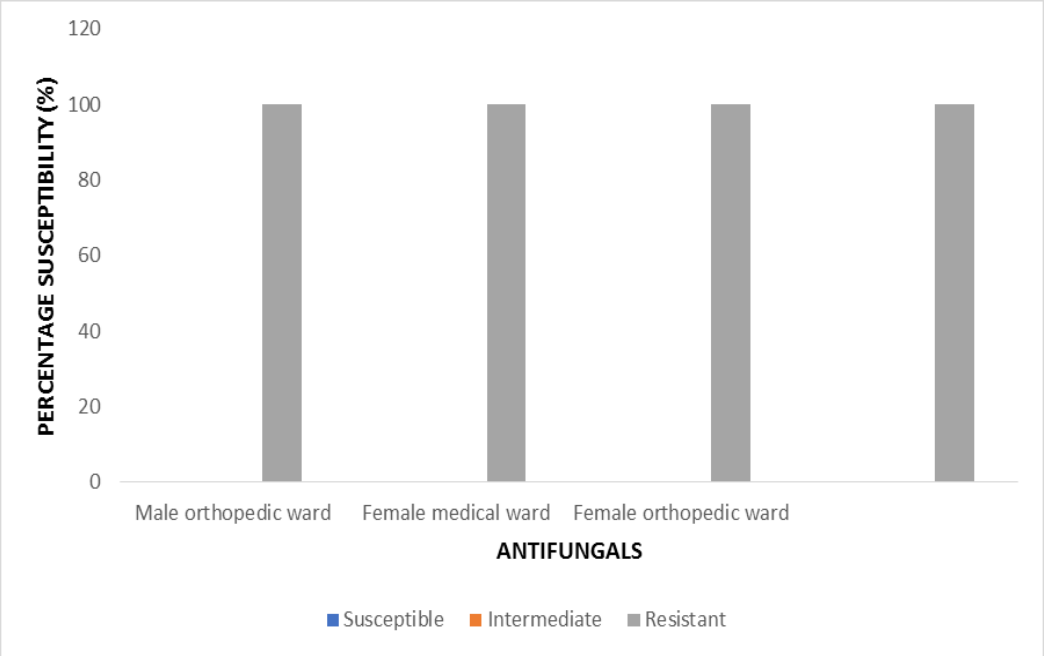

Fig.6b: Susceptibility pattern of *Penicillium chrysogenum* from various wards to fluconazole.

Fig 7 below shows that the age range of 56 to 75 years accounted for more of those with pressure injuries.

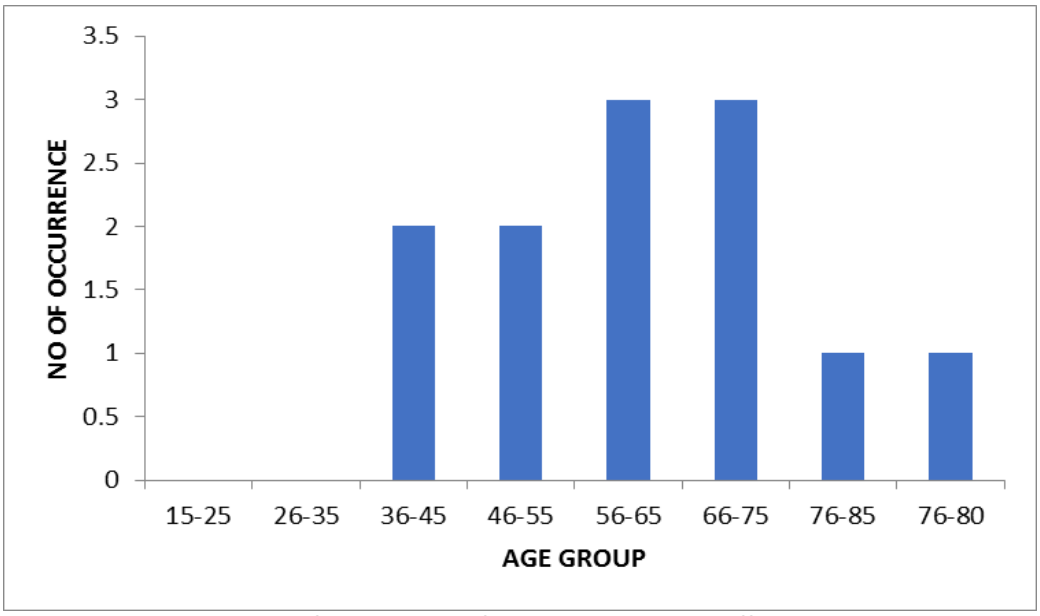

Fig.7: Frequency of occurrence of pressure ulcers in different age groups.

Pseudomonas aeruginosa was the most prevalent organism in pressure ulcers in our study followed by *Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli* accounting for 8(22.9%), 7(20%) and 5(14.3%) respectively. *Coagulase negative staphylococci* were the least prevalent bacteria accounting for 3(8.6%) of the total bacterial count. This is in agreement with a previous study conducted by Braga *et al* [21] which found Enterobacteriaceae to be the most commonly isolated group of bacteria (49%) colonizing patients with pressure ulcers, followed by *Staphylococcus aureus* (28%) and non-fermenting GNB (23%). Another study by Braga and colleagues [14] showed that the most commonly isolated microorganism was *Escherichia coli* (35.3%) followed by *Staphylococcus spp* (31.3%), *Enterobacter* (9.8%), *Pseudomonas aeruginosa* (3.9%), *Klebsiella pneumoniae* (3.9%), *Acinetobacter* (3.9%) and *Proteus mirabilis (2%).*In another study, Espejo *et al* [10] showed that the most frequently isolated microorganisms from the ulcer cultures were *Escherichia coli* and *Proteus spp*, followed by *Staphylococcus aureus, Bacteroides spp, Pseudomonas spp,* and *Enterococcus spp*. Another study conducted by Fazel *et al* [22] revealed that amongst all the isolates *Pseudomonas aeruginosa* had the highest frequency of 36% followed by *Staphylococcus aureus* (32%) and *Escherichia coli* (30%).

From the thirty-four bacterial isolates 24 (70.6%) were Gram negative whereas, 10 (29.4%) were Gram positive with *Pseudomonas aeruginosa* being the most prevalent Gram negative and *Staphylococcus aureus* the most prevalent Gram-positive organism. This is in line with the study and report conducted by [23] which showed that aerobic Gram-negative rods and aerobic Gram-positive cocci were the bacterial categories most commonly isolated. Anaerobes typically accounted for less than one third of bacterial isolates. The research work conducted by [24] showed Gram- positive organisms predominantly *Staphylococcus aureus* had more occurrence of 34(54.8%) than Gram negative organisms 28(45.2%).

In this study, 12 patients (100%) were detected to have ≥ 2 different bacteria in their pressure ulcers. The study conducted by Dolati and colleagues [22] showed that 17(34.6%) patients were detected to have more than two species of bacteria in the pressure ulcers with stage 3 or 4. Another research conducted by [10] showed that ulcer culture was polymicrobial in 41 cases (73.2%). *Aspergillus fumigatus* accounted for 50% of all 12 fungi isolated in our study.

The findings of our study revealed a high prevalence of multidrug resistance involving both Gram-negative and Gram-positive bacteria. A very striking observation was the nonsusceptibility of all *S. aureus* and other staphylococcal isolates to almost all tested antibiotics. Worthy of note is the fact that these isolates exhibited 100% resistance to Ceftriaxone (one of the commonly prescribed antibiotics in the emergency department), clindamycin and cefoxitin. All *Escherichia coli* isolates were susceptible to gentamicin while all *Klebsiella pneumonia* isolates were resistant to clindamycin, levofloxacin and erythromycin. Also interesting was the fact that whereas *Pseudomonas aeruginosa* was resistant to levofloxacin (a drug considered a traditional antipseudomonal drug) it was however almost 100 % susceptible to cefoxitin. Several other studies had been done, some with similar findings and others different [24-26].

The result of this study showed that *Pseudomonas aeruginosa* accounted for 34.3% of infected pressure ulcer, this is in line with studies conducted by[22] in which *Pseudomonas aeruginosa* accounted for 36% of the total isolates.

Pseudomonas aeruginosa is the most common pathogen in chronic wounds such as pressure ulcers and can cause delayed healing of ulcer because of its ability to form biofilms that are highly resistant to antimicrobial agents.[27]

The mean age in this study was 60 ± 13.3 years with 58.3% female and 41.7% male respectively. The gluteus region accounted for 50% of pressure ulcers in our study followed by the sacrum (16.67%) although [21] found the sacral region to be the commonest site of pressure ulcer in their study. The number of days taken to develop pressure ulcer depends on the diagnosis of disease state.[28] Geriatric patients were more likely to develop pressure ulcer due to the presence of co-morbidities as these multiple chronic conditions impair mobility. The duration of hospital stay has been somewhat linked to development of pressure ulcer as a study found that patients with a hospital stay of ≤ 6days rarely developed pressure ulcer.

All patients were admitted for clinical reasons which includes spinal cord injury with a percentage occurrence of 50%, followed by cardiovascular disease (33.33%), cancer and fracture (8.33%). A study conducted by [28] revealed that the direct risk factor associating pressure ulcer and cardiovascular diseases is primarily poor perfusion occurring in all medical settings. For heel ulcers, peripheral vascular disease and diabetes are particularly strong causal factors. Pressure ulcer may be unavoidable in patients with these conditions, given circumstances such as reduced mobility and undernutrition.

Out of the twelve patients with pressure ulcers, eleven were placed on systemic antibiotics some for long periods as shown in Tab 2 above. Prolonged administration of antibiotics will expectedly predispose to development of resistance. The findings of our study thus call to question the efficacy of these agents prescribed for prolonged periods.

Out of twelve patients with pressure ulcers, 11 patients had their ulcer dressed regularly (daily, two days interval and four days interval). According to a research work conducted by Boyko and colleagues [29] wound dressings should be changed regularly and as soon as they become soiled with urine or feces to prevent wound contamination.

There is an established relationship between the age of patient, duration of immobilization and incidence of pressure ulcers as seen in Fig 7 above.

CONCLUSION

Antimicrobial resistance is a major concern particularly in the health care setting. The misuse and overuse of these antibiotics has caused development of resistant strains of microorganisms. Although antibiotic use cannot be avoided completely due to the persistent occurrence of infectious diseases, there is however need to use them with caution to prevent development of resistant strains. There was no significant difference in the microbial population isolated from pressure injuries across the various wards in our study. There was, however, a significant difference in the antimicrobial susceptibility pattern of the various organisms across the various wards. There is therefore a need for continued surveillance and epidemiological studies to establish the peculiar susceptibility patterns of pathogens in various units to enhance the outcomes of antibiotic usage. Improved infection prevention and control mechanisms

such as proper cleaning and disinfection of hospital environment as well as reduction in hospital stay to minimize transmission of multidrug resistant organisms in the hospital are also recommended.

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