



## BACTERIOLOGICAL PROFILE OF WOUND INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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### Abstract:

**Introduction:** Wound infections are frequently attributed to several bacterial pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and members of Enterobacteriaceae family. With emergence of MRSA, ESBL producers and Carbapenem Resistance Producers, antibiotics have become indispensable in treating the infections making surveillance of changing prevalent microorganisms along with their antibiotic susceptibility patterns a need of the hour.

**Objective:** This study was aimed to isolate bacteria from wound infections and detect their antibiotic susceptibility pattern.

**Methodology:** A total of 100 samples from wounds were processed and Antibiotic susceptibility pattern was tested by Kirby Bauer Disc Diffusion method. ESBL production was tested by a combined disc diffusion assay, Carbapenem Resistance was tested using Meropenem discs and MRSA were detected using Cefoxitin disc.

**Results:** Culture positivity was 86% with predominant isolate being *Pseudomonas aeruginosa* (23.4%) followed by *Staphylococcus aureus* (21.2%) and *Klebsiella* species (17%). ESBL producers were 23.40% of the isolates, 51.06% isolates being Carbapenem Resistant and 19.19% being MRSA.

**Conclusion:** Regular surveillance, strict implementation of infection control practices and antibiotic policy added with root cause analysis of wound infections can reduce the burden of antibiotic resistance.

**Keywords:** Wound Infections, Antibiotic Resistance, Extended Spectrum Beta Lactamase (ESBL), Carbapenem Resistance, MRSA

### INTRODUCTION

The skin represents a defence barrier against the colonization of pathogens. The disruption of the normal anatomical structure by surgical operations or by chemical, physical, mechanical and thermal events, with an alteration of skin functions, results in a wound.<sup>1</sup> Skin when exposed to injuries, scratches and in contact with the external environment, there is loss of integrity and the added moisture acts as a nutritive environment for the microorganisms to colonize and proliferate delaying the healing process.<sup>2,3,4,5</sup> Skin and soft tissue infections contribute to higher rates of morbidity and mortality affecting the quality of life.<sup>2,6</sup>

Wounds are divided into two categories: Acute and Chronic. Acute wounds like cuts, burns, abrasions and surgical wounds heal through the regular phases of wound repair.<sup>6</sup> Chronic wounds can

be exacerbated by predisposing factors like advancing age, obesity, metabolic disorders like Diabetes mellitus, immunosuppression, poor nutrition. Leg & foot ulcers, pressure sores as a consequence of impaired arterial supply or venous drainage are hypoxic, leading to necrosis and cell death, making it ideal for the wound microflora to proliferate.<sup>5</sup>

In the initial stage of the infection process, most common causative organisms involved are *Staphylococcus aureus*, *Streptococcus pyogenes*. When chronic wound is developed, gram negative organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter* species, *Proteus* species play a role.<sup>2, 7, 8</sup> The risk of surgical wound infection is 1-5% of post operative wound infections in case of clean surgeries and 27% risk in dirty procedures and can have a polymicrobial etiology.<sup>5, 9</sup> Fungi like *Candida* species, *Aspergillus fumigatus* also play a role due to their widespread nature and even as part of normal flora.<sup>10</sup>

The polymicrobial wound infections will add up the chances of not only genotypic resistance, but also phenotypic resistance or antimicrobial tolerance.<sup>11, 12</sup>

There is a need for careful and up to date monitoring of the changing trends of the pathogens and the identification of antimicrobials to which they are susceptible for initiating effective pathogen specific treatment and combating antimicrobial resistance at the same time reducing the patient morbidity and mortality.<sup>12</sup> The study was aimed to isolate bacteria from wound infections and detect their antibiotic susceptibility pattern.

## METHODOLOGY

**Ethical Consideration:** Institutional Ethical Clearance was obtained prior to the study.

**Source of Clinical Samples:** Clinical samples from wound infections (Swab, Pus, Abscess fluid, Tissue) received in the Microbiology laboratory for culture and sensitivity testing

**Study Design:** Prospective study over a period of 2 months.

**Sample Size:** 100 samples

**Inclusion Criteria:** Samples from wound infection

**Exclusion Criteria:** Samples not from wound infection

### Method:

#### Isolation of bacteria from wound infections:

- Clinical samples from wound infections received by the Microbiology laboratory for culture and sensitivity testing were inoculated on Nutrient agar, Blood agar and Mac Conkey agar. The culture plates were incubated at 37°C for 24–48 hours. Once the growth is obtained, bacteria were isolated based on morphology and gram stain

#### Detection of the antibiotic susceptibility pattern:

- Identification and Antibiotic susceptibility tests were performed by Kirby Bauer Disc Diffusion Method based on Clinical and Laboratory Standards Institute (CLSI) guidelines.

- The following antibiotics were tested for Antibiotic Susceptibility Testing for Gram Negative Bacilli

- Enterobacteriaceae: Ceftazidime, Cefoxitin, Ciprofloxacin, Gentamicin, Amikacin, Piperacillin / Tazobactam, Imipenem, Meropenem, Cotrimoxazole,

- Non – Fermenters: Ceftazidime, Cefoxitin, Ciprofloxacin, Gentamicin, Amikacin, Piperacillin / Tazobactam, Imipenem, Meropenem, Tobramycin, Aztreonam

- The following antibiotics were tested for Antibiotic Susceptibility Testing for Gram Positive Cocci:

- Penicillin, Cefoxitin, Ceftriaxone, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Amikacin, Cotrimoxazole, Linezolid

#### Detection of ESBL production:

- Isolates resistant to Ceftazidime (inhibition zone <17mm) by Disc Diffusion method were considered as potential ESBL producers and tested further

- The Ceftazidime resistant strains were tested for ESBL production by a combined disc diffusion assay using Ceftazidime disc and Ceftazidime / Clavulanic acid disc
- The zone diameter difference of >5 mm around the Ceftazidime / Clavulanic acid disc in comparison to the zone size of the Ceftazidime disc, was confirmed as ESBL producer.

Detection of Carbapenem Resistance:

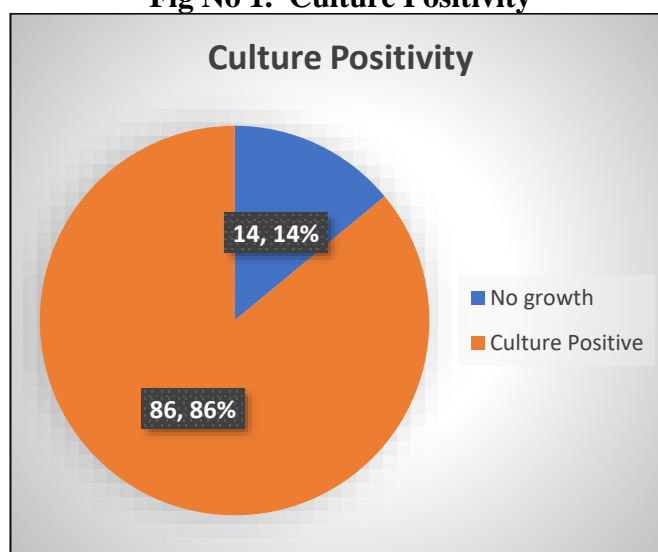
- Isolates resistant to Imipenem and Meropenem (inhibition zone <19mm) by Disc Diffusion method were considered Carbapenem Resistant Strains

Detection of MRSA:

- Isolates resistant to cefoxitin with zone of inhibition <19mm by Disc Diffusion Method were reported as MRSA

**RESULTS**

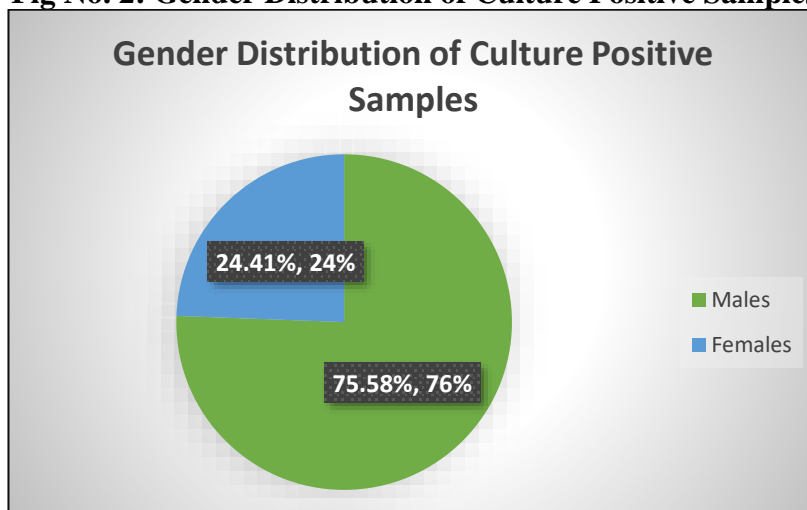
**Fig No 1. Culture Positivity**



**Table No. 1: Sample wise organism isolation**

Samples with	Number (%)
Single organism isolated	77 (89.53%)
Polymicrobial isolated	9 (10.46%)
Total	86 (100%)

**Fig No. 2: Gender Distribution of Culture Positive Samples**



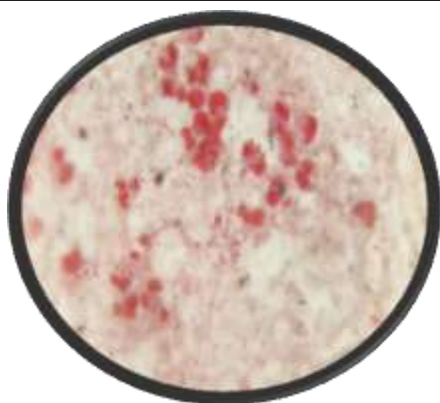
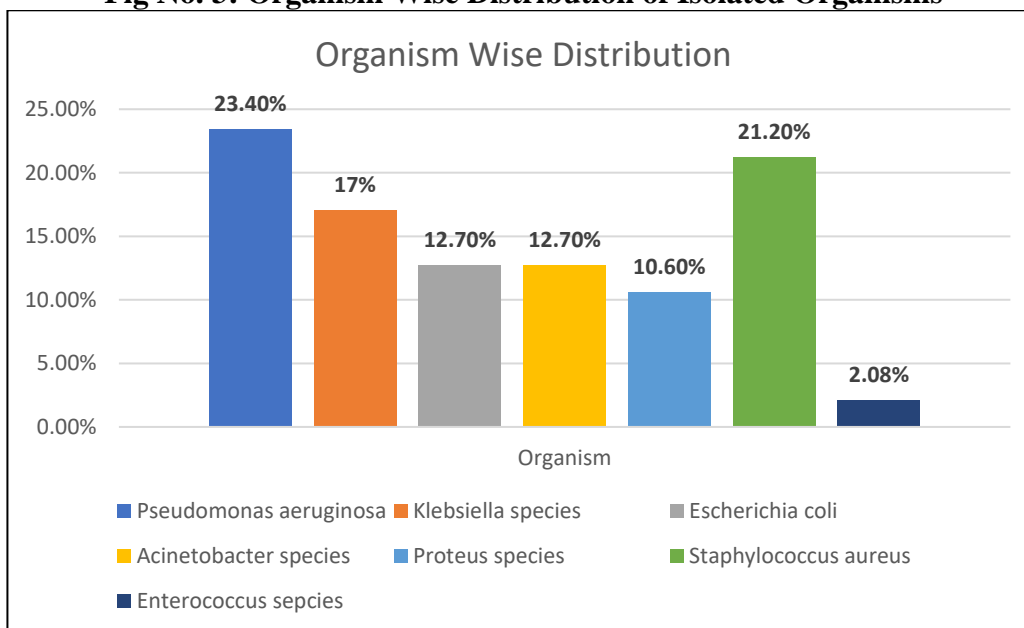
**Table No. 2: Age Distribution of Culture Positive Samples**

Age (in years) Distribution	Number (%)
<1 – 20	8 (9.30%)
21 – 40	24 (27.90%)
41 – 60	28 (32.55%)
61 – 80	24 (27.90%)
81 - 100	2 (2.30%)
Total	86 (100%)

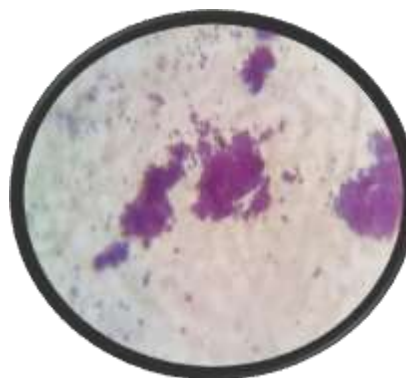
**Table No. 3: Department Wise Distribution of Samples**

Department	Number (%)
General Surgery	50 (50%)
ENT	24 (24%)
Orthopedics	6 (6%)
Urology	6 (6%)
ICU / Stepdown	6 (6%)
Dermatology	2 (2%)
General medicine	2 (2%)
Cardiothoracic and vascular surgery (CTVS)	2 (2%)
Pediatrics	2 (2%)
Total	100 (100%)

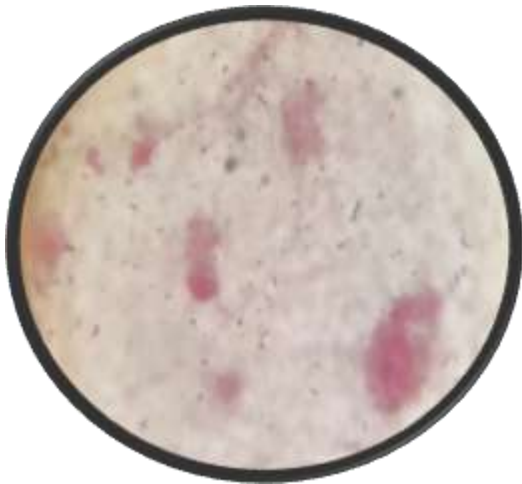
**Fig No. 3: Organism Wise Distribution of Isolated Organisms**



**Fig 4: Pus cells with Gram Negative Bacilli**



**Fig 5: Gram Positive Cocci**



**Fig 6: Pus cells with Gram Negative Bacilli and Gram Positive Cocci**



**Fig 7: Beta Hemolytic colonies on Blood agar**



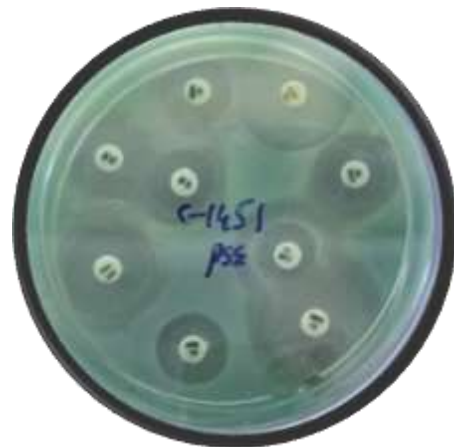
**Fig 8: Lactose Fermenting Colonies (Mucoid - *Klebsiella pneumoniae*)**



**Fig 9: Lactose Fermenting Colonies (*Escherichia coli*)**



**Fig 10: Green colored colonies on Nutrient Agar (*Pseudomonas aeruginosa*)**



**Fig 11: Antibiotic Susceptibility Testing**

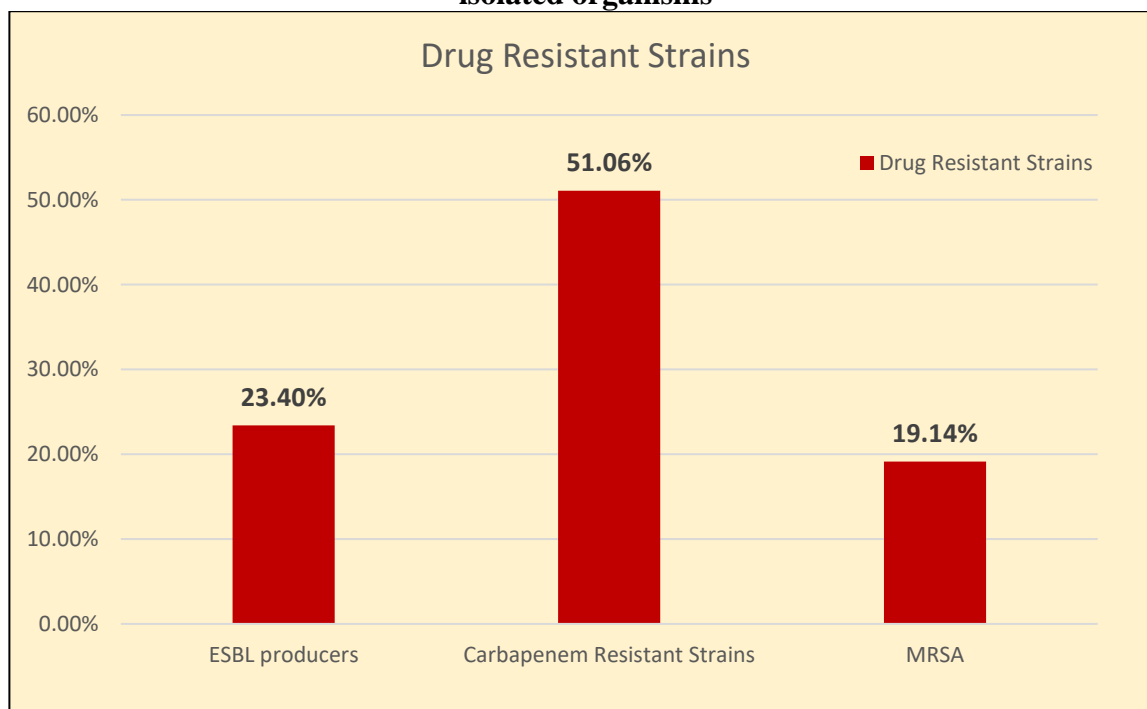
**Table No. 4: Antibiotic Susceptibility Pattern of Gram-Negative Isolates**

Antibiotics	Escherichia coli (n=12)	Klebsiella species (n=16)	Pseudomonas aeruginosa (n=22)	Acinetobacter species (n=12)	Proteus vulgaris (n=10)
Ceftazidime	2 (16.6%)	10 (62.5%)	10 (45.4%)	4 (33.3%)	4 (40%)
Cefoxitin	0 (0%)	2 (12.5%)	0 (0%)	2 (16.6%)	0 (0%)
Ciprofloxacin	6 (50%)	12 (75%)	14 (63.6%)	8 (66.6%)	10 (100%)
Gentamicin	6 (50%)	6 (37.5%)	8 (36.3%)	2 (16.6%)	6 (60%)
Amikacin	4 (33.33%)	2 (12.5%)	6 (27.2%)	2 (16.6%)	4 (40%)
Piperacillin / Tazobactam	4 (33.33%)	4 (25%)	18 (81.8%)	10 (83.3%)	8 (80%)
Imipenem	2 (16.6%)	4 (25%)	10 (45.4%)	8 (66.6%)	2 (20%)
Meropenem	4 (33.33%)	0 (0%)	2 (9.09%)	4 (33.33%)	4 (40%)
Cotrimoxazole	6 (50%)	8 (50%)	2 (9.09%)	6 (50%)	10 (100%)
Tobramycin	NA	NA	16 (72.7%)	NA	NA
Aztreonam	NA	NA	6 (27.2%)	NA	NA

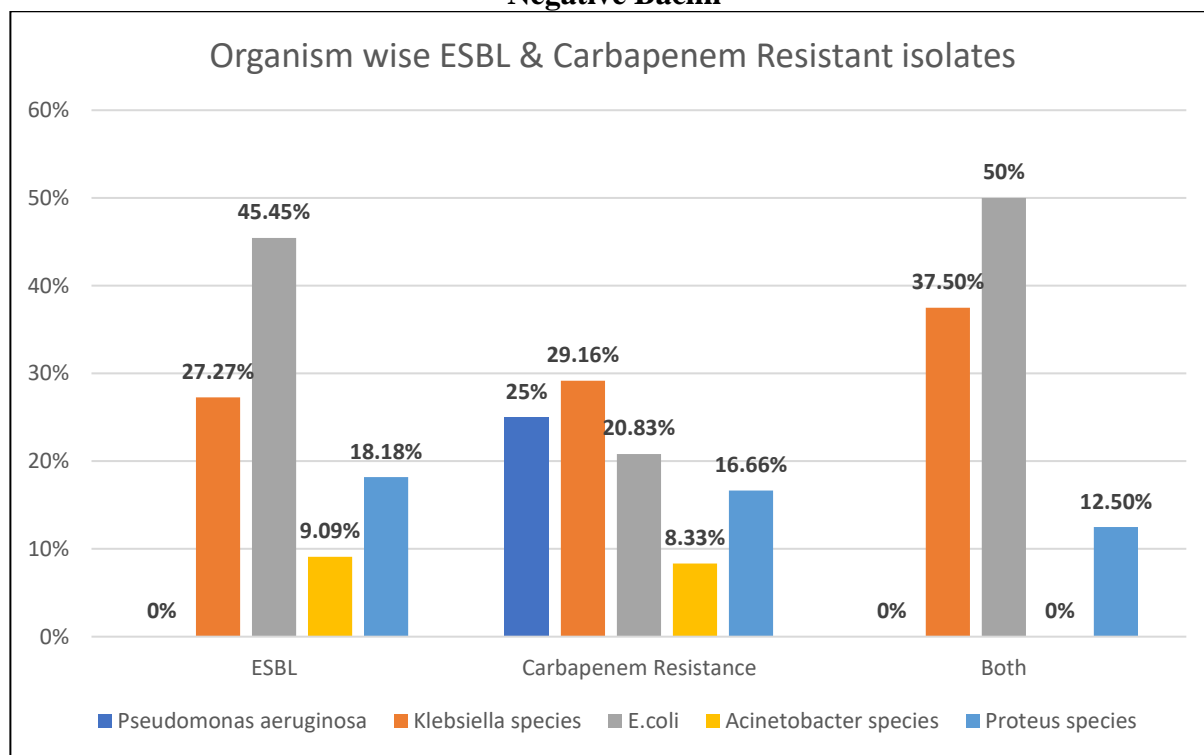
**Table No. 5: Antibiotic Susceptibility Pattern of Gram-Positive Isolates**

Antibiotics	Staphylococcus aureus (n=20)	Enterococcus species (n=2)
Penicillin	2 (10%)	0 (0%)
Cefoxitin	2 (10%)	NA
Ciprofloxacin	8 (40%)	0 (0%)
Cotrimoxazole	12 (60%)	NA
Gentamicin	8 (40%)	NA
High level gentamicin	NA	0 (0%)
Amikacin	4 (20%)	0 (0%)
Erythromycin	0 (0%)	NA
Clindamycin	8 (40%)	NA
Linezolid	14 (70%)	NA
Teicoplanin	NA	0 (0%)
Vancomycin	NA	2 (100%)

**Fig No 12: ESBL producers, Carbapenem Resistant and MRSA isolates Distribution among isolated organisms**



**Fig No 13: Organism wise ESBL & Carbapenem Resistant isolates Distribution among Gram Negative Bacilli**



## DISCUSSION

The present study was carried out in the Department of Microbiology over a period of 2 months. Among the 100 samples received, 86 samples showed culture positivity (86%) and 14 samples showed no growth (14%). Similar culture positive rates were reported by Abdu et al<sup>13</sup> (83.03%), Aynalem Mohammed et al<sup>14</sup> (83.9%)

Among the 86 culture positive samples, 77 samples (89.53%) isolated single organism and 9 (10.46%) samples isolated more than 1 organism with a total of 94 isolates. Similar results of isolation were reported by Puca et al<sup>15</sup> as single organism (75.3%) and polymicrobial infection as 24.7%. Similar polymicrobial growth was observed by Aynalem Mohammed et al<sup>14</sup> (18.3%).

There was a male preponderance (75.59%) among the culture positive samples similar to Abdu et al (56.15%). Of the 86 culture positive samples, maximum was among the age group 41 – 60 years (32.55%), followed by 21 – 40 & 61 – 80 years (27.90%). This might be supported by the fact that males are more predisposed to trauma due to their occupations like riders, farming, industry supported by the most isolated organisms were from ages 41 – 60 years which is the working population as also mentioned by Abdu et al<sup>13</sup>.

Various samples were received like pus, discharge from wound infection, debrided tissue from a wound infection, pus from abscess, cyst, ulcer, drain site discharge, swab from cellulitis Maximum samples received from the Department of General Surgery (50%), followed ENT (24%), Orthopedics, Urology, ICU & Stepdown (6%).

Among the 94 isolates, among the Gram-negative bacilli, *Pseudomonas aeruginosa* was most commonly isolated organism (23.4%) followed by *Klebsiella species* (17%) and *Escherichia coli* & *Acinetobacter species* (12.7%). Among the Gram-positive isolates maximum isolates were *Staphylococcus aureus* (21.2%). M. M. Alam et al<sup>16</sup> also reported *Pseudomonas aeruginosa* as the most commonly isolated organism among Gram negative bacilli (27.1%), followed by *Escherichia coli* (26.2%) and *Klebsiella* (14.9%) similar to our study, and *Staphylococcus aureus* as the most commonly isolated organism among Gram positive organisms (75.9%) (35.99% among all isolates) similar to our study. Similar findings were reported by Abdu et al<sup>26</sup> with *Pseudomonas aeruginosa* being the most predominant organisms (17.07%), followed by *Escherichia coli* (11.58%) and

*Klebsiella pneumoniae* (10.37%). Ahmed A. Al – Naqshbandi et al<sup>17</sup> also reported gram negative isolates as the predominant organisms isolated from wounds similar to our study. Ahmed A. Al – Naqshbandi et al<sup>17</sup> reported *Staphylococcus aureus* as the most commonly isolated Gram - positive organism (23.94%) similar to our study.

On the contrary, M. E. Abalaka et al<sup>18</sup> reported *Staphylococcus aureus* as the most commonly isolated organism (35%) followed by *Pseudomonas aeruginosa* (26.7%). On the contrary M. E. Abalaka et al<sup>18</sup> reported *Escherichia coli* as least occurring organism (3.3%) while our study reports *Enterococcus* species as the least occurring organism (2.08%). The Extracellular adherence protein (Eap) of *Staphylococcus aureus* plays a key role in delayed wound healing blocking angiogenesis and slowing the inflammatory response as reported by Abalaka et al<sup>18</sup>.

In this study, *Escherichia coli* showed maximum susceptibility to Ciprofloxacin, Gentamicin and Cotrimoxazole (50%), least sensitivity was shown for Ceftazidime and Imipenem (16.6%). No susceptibility was shown for Cefoxitin (0%). *Klebsiella* species showed maximum sensitivity to Ciprofloxacin (75%), followed by Ceftazidime (62.5%) and Cotrimoxazole (50%). Lower sensitivity was observed towards Piperacillin / Tazobactam and Imipenem (25%). Least susceptibility was shown to Cefoxitin, Amikacin (12.5%). No isolate showed susceptibility to Meropenem (0%). Similar susceptibility patterns towards Cephalosporins (40%) and ciprofloxacin (30 – 50%) were reported by Afroz et al<sup>19</sup>, Esebelahie, N. O et al<sup>20</sup> (Ciprofloxacin 58.95%, Gentamicin 32.5%. Aynalem et al<sup>14</sup> also reported most of the *Escherichia coli* isolates showing susceptibility to gentamicin (87.5%), Chloramphenicol (75%) and ciprofloxacin (62.5%) and *Klebsiella* species showing susceptibility to gentamicin (70.6%), followed by cephalosporins (47.1%).

*Acinetobacter* species showed maximum susceptibility to Piperacillin / Tazobactam (83.3%) followed by Ciprofloxacin, Imipenem (66.6%) and Cotrimoxazole (50%). Lower susceptibility rates were observed towards Ceftazidime and Meropenem (33.33%) followed by Cefoxitin, Gentamicin, Amikacin (16.6%). *Proteus vulgaris* showed maximum susceptibility to Ciprofloxacin and Cotrimoxazole (100%), followed by Piperacillin / Tazobactam (80%), followed by Gentamicin (60%). Lower susceptibility was observed towards Ceftazidime, Amikacin and Meropenem (40%). Least susceptibility was shown to Imipenem (20%) and no sensitivity towards Cefoxitin (0%). Alam et al<sup>16</sup> reported that piperacillin / tazobactam and carbapenems had a better activity against *Proteus* and low resistance against carbapenems contrary to our study.

*Pseudomonas aeruginosa* showed highest susceptibility towards Piperacillin / Tazobactam (81.8%), followed by Tobramycin (72.7%), Ciprofloxacin (63.6%). Lower susceptibility rates were observed for Ceftazidime and Imipenem (45.4%), followed by Gentamicin (36.3%), Aztreonam and Amikacin (27.2%). Least susceptibility was observed towards Meropenem and Cotrimoxazole (0.09%). High susceptibility to Piperacillin / Tazobactam corroborates the reported study by Abdu et al<sup>13</sup> (96.43%) and Amoran O. E et al<sup>21</sup>. Fluoroquinolones and Gentamicin were more effective in this study which correlates with Esebelahie, N. O et al<sup>20</sup>. The findings by Aynalem et al<sup>14</sup> reported sensitivity to Aminoglycosides similar to the findings of this study.

Among the 20 *Staphylococcus aureus* isolates, maximum susceptibility was observed to Linezolid (70%), followed by Cotrimoxazole (60%), Ciprofloxacin, Gentamicin and Clindamycin (40%), Amikacin (20%). Least susceptibility was observed against Penicillin & Cefoxitin (10%) and Erythromycin (0%). Similar high susceptibility to linezolid was reported by Alam et al (99%)<sup>16</sup>. No Vancomycin resistant *Enterococci* were observed in this study similar to the findings reported by Alam et al<sup>16</sup>.

Among the 94 isolates, 23.40% (22) isolates are ESBL producers, 51.06% isolates (48) are Carbapenem Resistant isolates and 19.14% (18) are MRSA. Alam et al<sup>16</sup> reported 34.16% MRSA isolates in their study correlating with such numbers in our study. Higher rates of MRSA were reported by Aynalem et al<sup>14</sup> (76.9%).

Among the 22 ESBL producers, 45.45% (10) were *Escherichia coli*, 27.27% (6) isolates were *Klebsiella* species, 18.18% (4) isolates were *Proteus* species and 9.09% (2) isolates were *Acinetobacter* species. Roopashree et al<sup>22</sup> reported majority of the ESBL producers were *Escherichia*



coli (50.90%) followed by *Klebsiella pneumoniae* (18.18%) similar to the ESBL producers isolates from our study.

Among the Carbapenem resistant isolates, 29.16% (14) isolates were *Klebsiella* species, 25% (12) were *Pseudomonas aeruginosa*, 20.83% (10) isolates were *Escherichia coli*, 16.66% (8) were *Acinetobacter* species and 8.33% (4) were *Proteus* species. Roopashree et al<sup>22</sup> reported higher rates of Carbapenem resistance among *Pseudomonas aeruginosa* followed by *Acinetobacter* species, *Escherichia coli* and *Klebsiella* species contrary to our study.

The difference in antibiogram could be due to the difference in the bacterial strains among the population, difference in the infection control measures, variation in the wounds and surgical techniques.

Of the 16 isolates which were both ESBL producers and Carbapenem Resistant, 50% (8) were *Escherichia coli*, 37.50% (6) were *Klebsiella* species and 12.5% were *Proteus* species. Such alarming multidrug resistance among gram negative bacilli is limiting the treatment options for the patients. Carbapenems are a reliable set of drugs for treating bacterial infections, and such emerging resistance patterns raises the issue of major public health concern. Such changing trends have to be frequently monitored so that the right antibiotic can be given to the patient for better patient care and reducing morbidity and mortality.

### **CONCLUSION:**

Cost effective and simple methods for diagnosis can be incorporated in routine laboratory for improving clinical management of the patients suffering from infections caused by drug resistant organisms. Increasing proportion of antibiotic resistance is implicating persistence of drug-resistant infections leading to higher morbidity and mortality posing a serious health threat rendering treatment to such infections a high challenge. Timely reporting of drug-resistant strains will help in preventing the spread of multidrug resistance isolates. Regular surveillance with root cause analysis of all wound infections, strict adherence to hospital antibiotic policy, implementing strict infection control practices must be considered to reduce the wound infection rates and also reduce the burden of antibiotic resistance.

### **ACKNOWLEDGEMENT:**

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### **AUTHORS CONTRIBUTIONS:**

Tamma Saisashank Reddy worked as principal investigator and contributed to the drafting the proposal, conducting the research, collect the data. M. Shabnum worked as a coinvestigator and played key role in acquiring the approval for the study, reviewing the content, analyzing the data and its interpretation, drafting the manuscript and preparing for its publication. All authors have read and approved the final manuscript.

### **COMPETING INTERESTS:**

None

### **AUTHORS FUNDING:**

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