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Original Article

PREVALENCE AND PHENOTYPIC CHARACTERIZATION OF MULTI-DRUG-RESISTANT ISOLATES CAUSING WOUND INFECTIONS IN A TERTIARY CARE CENTRE

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ABSTRACT

Objective: Wound infections lead to 70-80% mortality among post-surgeries and one-third of nosocomial infections. The prolonged hospitalization due to diagnostic tests, complete antibiotic course, and clearing of wound infection all together increase the healthcare cost.

Methods: The present study was a cross-sectional study carried out in the Department of Microbiology, Central laboratory, and teaching hospital from May 2022 to October 2022. All consecutive, non-duplicate gram-positive and gram-negative bacteria isolates were collected from pus and wound swabs from outpatients and hospitalized patients during the study period.

Results: A total of 260 isolates from various wound swabs and pus samples were collected from March 2022 to August 2022. Species-wise distribution of organisms along with antibiotic susceptibility testing shows that 15 out of 63 (24%) *Escherichia coli,* 12 out of 38 (31.5%) *Klebsiella pneumoniae*, 06 out of 29 (20.6%) *P. aeruginosa,* 06 out of 09 (40%) *Acinetobacter baumannii*, 05 out of 08 (62%) *Klebsiella oxytoca*, 04 out of 12 (33%) *Citrobacter freundii*, 01 out of 07 (14.3%) *Enterobacter aerogenes* were multi-drug-resistant (MDR). Previously few studies mentioned *S. aureus* was predominant, followed by *P. aeruginosa* in polymicrobial wound infections. Our study found that around 2-3% of cultures showed two organisms. The antibiotics like amikacin and imipenem worked well against all gram-negative organisms up to 72%, and 85%, respectively. Similar findings of organisms in other studies showed sensitivity to amikacin and imipenem up to 77% and 100%; 70% and 83%, respectively.

Conclusion: The organisms causing wound infections and the empirical therapy and switch to correct antibiotics as soon as possible to avoid misuse of antimicrobials and prevent the spread of drug-resistant strains among the community and hospital setup.

Keywords: Prevalence, Wound infection, Surgical site infection

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INTRODUCTION

The important organ in our body that defends the colonization of pathogens is skin. Therefore, the wound results from the breach in the normal anatomical structure and functions through surgeries or mechanical, disinfectants, antisepsis, and iatrogenic [1]. The wound healing and repair will be through regular phases in case of cuts, burns, abrasions, and surgical wounds. The wound healing rate and quality of life are compromised in the case of infected damage [2]. Wound infections lead to 70-80% mortality among post-surgeries and one-third of nosocomial diseases. The morbidity and mortality are more regardless of the type of wound because of Wound infections, especially in developing countries. The prolonged hospitalization due to diagnostic tests, complete antibiotic course, and clearing of wound infection all together increase the healthcare cost [2-5]. In association with diabetes or immunodeficiency diseases leads to delay in wound healing in chronic wounds like arterial and trophic ulcers [6].

Managing wound infections comprises two important aspects: wound care and antibiotic therapy [7]. The identification of microbial associated with wound infection, application of empirical antibiotics without sensitivity, and switch over to appropriate antibiotics after antibiotic sensitivity reports are helpful in the prevention of the spread of drug resistance, especially in hospitalized patients, which can reduce the healthcare cost [8, 9]. The organisms like Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Proteus species, Enterococcus faecalis, and Acinetobacter baumannii. During this first week, wound colonization is associated with Staphylococcus aureus followed by coagulase-negative Staphylococcus (CONS). Second-week wound colonization is associated with Pseudomonas and other gram-negative organisms [10, 11].

The ability to treat bacterial infections is critical in modern medicine. Without the help of antibiotics; many procedures in clinical settings would become unthinkable due to the risk of infections. Society has taken specific steps to restore antibiotic effectiveness like restrictions in use, antimicrobial stewardship and application of infection control guidelines. But unfortunately, the development of newer antibiotics and research and story for it has dwindled for various reasons [12, 13]. Control of hospital-acquired infections caused by multi-drug resistant Gram-negative bacilli is a significant problem. This led to therapeutic introduction of newer broad-spectrum antibiotics in hospitals, resulting in a subsequent increase in infections due to strictly aerobic Gram-negative bacilli, including Pseudomonas aeruginosa, Stenotrophomonas maltophilia and Acinetobacter species [13, 14].

Hospitals worldwide continue to face a crisis in the upsurge and dissemination of antimicrobial-resistant bacteria, mainly due to NFGNB causing nosocomial infections. The increasing prevalence of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* organisms are alarming, as effective antibiotics are severely limited. There is evidence of a causal link between antibiotic consumption and resistance. Other factors such as inter-hospital transfer of patients, community contribution to resistance, structures of health care systems, infection control practices and policies may play a significant role in determining the prevalence of resistance in a hospital [15-17]. So the prevalence and phenotypic characterization of multi-drug-resistant isolates causing wound infections in a tertiary care centre guides society about its management.

MATERIALS AND METHODS

Study design and sample processing

The present study was a cross-sectional study carried out in the Department of Microbiology, Central laboratory, and teaching

hospital from May 2022 to October 2022 [IRC/2022/343]. All consecutive, non-duplicate gram-positive and gram-negative bacteria isolates were collected from pus and wound swabs from outpatients, and hospitalized patients were included during the study period. All other samples were excluded from this study. The phenotypic identification of various isolates was made taking into account the colony morphology, pigment production, and other biochemical tests. The sugar reactions, TSI, fermentation of sugars, indole, urea, citrate, motility, and glucose oxidative fermentative test (OF test), etc. [fig. 1, 2] were done for speciation of the Gram-positive and Gram-negative bacteria according to standard protocol.

Antimicrobial susceptibility

The antimicrobial susceptibility of all isolates was done by the Kirby-Bauer disk diffusion method according to the CLSI guidelines 2021. Susceptibility was done using Amikacin 30 µg, Amoxicillin/Clavulanic 20/10 µg, Ceftazidime 30 µg, Ciprofloxacin 5 µg, Colistin sulphate 10 µg, Cefotaxim 30 µg, Cefepime 30 µg, Nitrofurantoin 300 µg, Levofloxacin 5 µg, Sulphamethoxazole/ Trimethoprim 25 µg under standard conditions [fig. 3].

Statistical analysis

All the data in the present study was entered into a spreadsheet (Excel 2019; Microsoft) for analysis. The Unpaired student's t-test was used as a test of significance for quantitative variables and a Chi-square test for qualitative variables. Yate's correction was applied to the Chi-square test whenever the frequency of the variable was less than 5. All tests were two-tailed, and a p-value<0.05 was taken as significant.

All tests were done using online GraphPad software. http://www.graphpad.com/quickcalcs/contingency2/ and http://www.graphpad.com/quickcalcs/ttest2/.

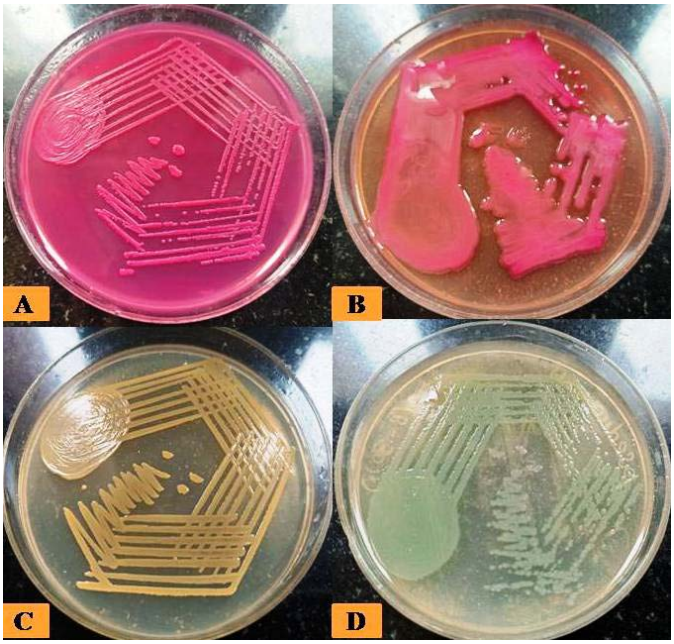

Fig. 1-1A: Flat lactose fermenting colonies of *Escherichia coli*; 1B: Mucoid lactose fermenting colonies of *Klebsiella pneumoniae*; 1C: Golden yellow pigment producing colonies of *Staphylococcus aureus*; 1D: Bluish-green pigment producing colonies of *Pseudomonas aeruginosa*

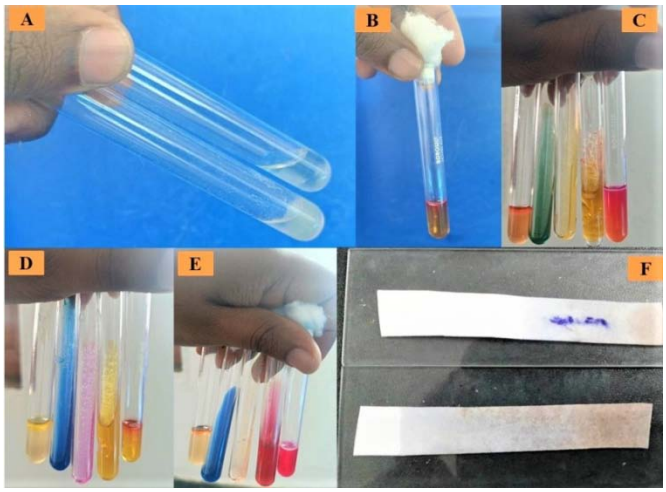

Fig. 2: Biochemical reaction of organisms, 2A-positive tube coagulase by *Staphylococcus aureus*; 2B-mannitol fermenting *Staphylococcus aureus*; 2C-Indole positive, citrate not utilized, urea not hydrolyzed, acid/acid with gas production in tsi, motile in mannitol motility medium by *Escherichia coli*; 2D-Indole negative, citrate utilized, urea hydrolyzed, acid/acid with gas production in tsi, non-motile in mannitol motility medium by *Klebsiella pneumoniae*; 2E-Indole negative, citrate utilized, urea not hydrolyzed, alkaline/no change in tsi, motile in mannitol motility medium by *Pseudomonas aeruginosa*; 2F-Oxidase test positive (*Pseudomonas aeruginosa*) and negative (*Acinetobacter baumannii*)

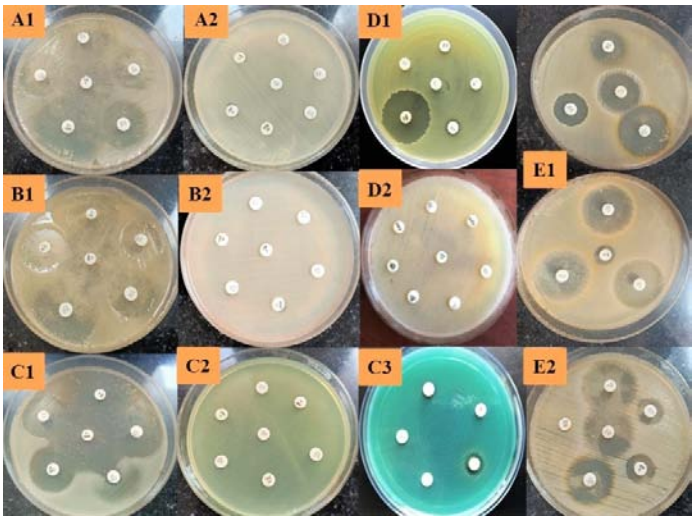

Fig. 3: Antibiotic susceptibility testing-$3A_1$: Sensitive *Escherichia coli*; $3A_2$: resistant *Escherichia coli*; $3B_1$: Sensitive *Klebsiella pneumoniae*; $3B_2$: Resistant *Klebsiella pneumoniae*; $3C_1$: Sensitive *Pseudomonas aeruginosa*; $3C_2$: Resistant *pseudomonas aeruginosa*; $3C_3$: Imipenem resistant *Pseudomonas aeruginosa*; $3D_1$: IMIPENEM SENSITIVE *Acinetobacter baumannii*; $3D_2$: Resistant *Acinetobacter baumannii*; $3E_1$: Sensitive *Staphylococcus aureus*; $3E_2$: Methicillin-resistant *Staphylococcus aureus* (MRSA) Showing resistance to cefoxitin

RESULTS

A total of 260 isolates from various wound swabs and pus samples were collected from March 2022 to August 2022. All isolates were screened for drug susceptibility by the Kirby-Bauer disk diffusion method, and the results were interpreted according to CLSI guidelines 2021. In this study, the Male: Female distribution of the sample was 172:88 (males: 66%; Females: 34%) [fig. 4]. For example, age distribution shows that most of the samples were from the age group 46-60 y followed by>60 y. [fig. 5, table 1]. Ward-wise distribution show that most of the samples in the present study were received from Surgery ICU 108 (41%) followed by Postoperative ward 72 (28%), surgery ward 53 (20%), and allied wards 27 (11%) [table 2, fig. 8]. Species-wise distribution of organisms causing wound infection shows that the most common isolate collected during the present study was *Escherichia coli* 63 (24%) followed by *Staphylococcus aureus* 49 (18.8%), *Klebsiella pneumoniae* 38 (15.1%), *Pseudomonas aeruginosa* 29 (11.3%), *Acinetobacter baumannii* 15 (5.78%), *Citrobacter freundii* 12 (4.5%), *Klebsiella oxytoca* 08 (3.04%), *Enterobacter aerogenes* 08 (3.04%), *Enterococcus species* 07 (2.6%), *Proteus mirabilis* 06 (2.3%), *Streptococcus pyogenes* 02 (0.78%), *Proteus species* 01 (0.39%), *Streptococcus viridans* 01 (0.39%) [table 3, fig. 7]. Species-wise distribution of organisms along with antibiotic susceptibility testing shows that 15 out of 63(24%) *Escherichia coli,* 12 out of 38 (31.5%) *Klebsiella pneumoniae*, 06 out of 29 (20.6%) *P. aeruginosa,* 06 out of 09 (40%) *Acinetobacter baumannii*, 05 out of 08 (62%) *Klebsiella oxytoca*, 04 out of 12 (33%) *Citrobacter freundii*, 01 out of 07 (14.3%) *Enterobacter aerogenes* were multi-drug-resistant (MDR) [table 4, fig. 8].

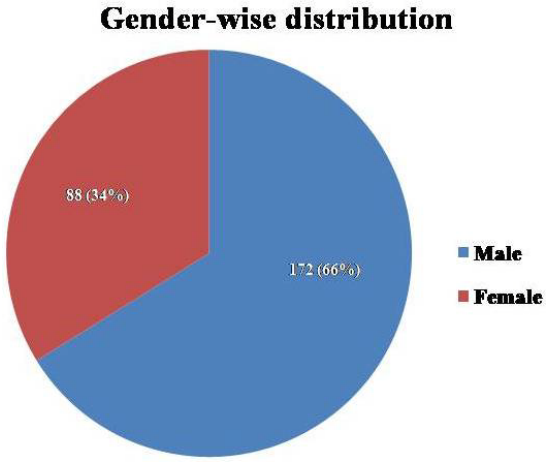

Fig. 4: Gender-wise distribution of wound infections

Table 1: Age-wise distribution of all samples (numbers and percentage)

Age in years (range)	Number	Percentage
0-15	10	4
16-30	23	8
31-45	53	21
46-60	112	43
>60	62	24
Total	260	100

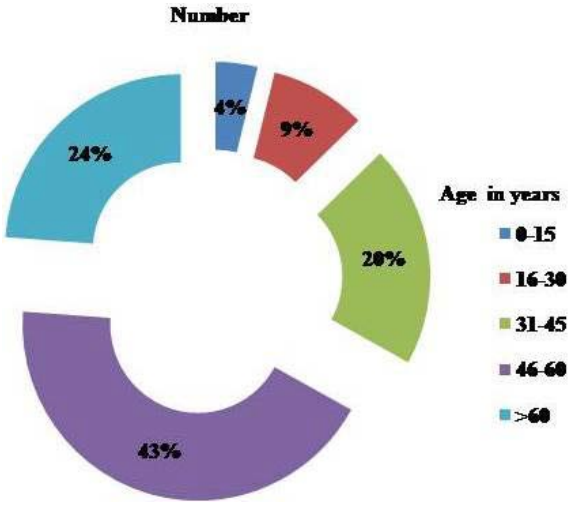

Fig. 5: Age-wise distribution of wound infections

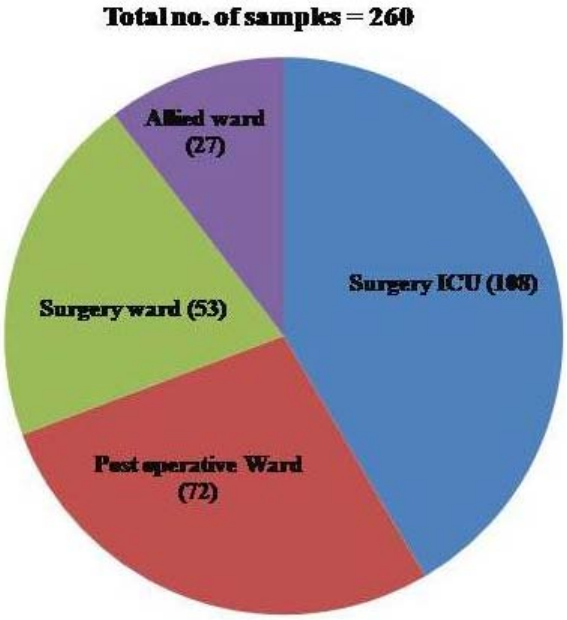

Fig. 6: Ward-wise distribution of sample

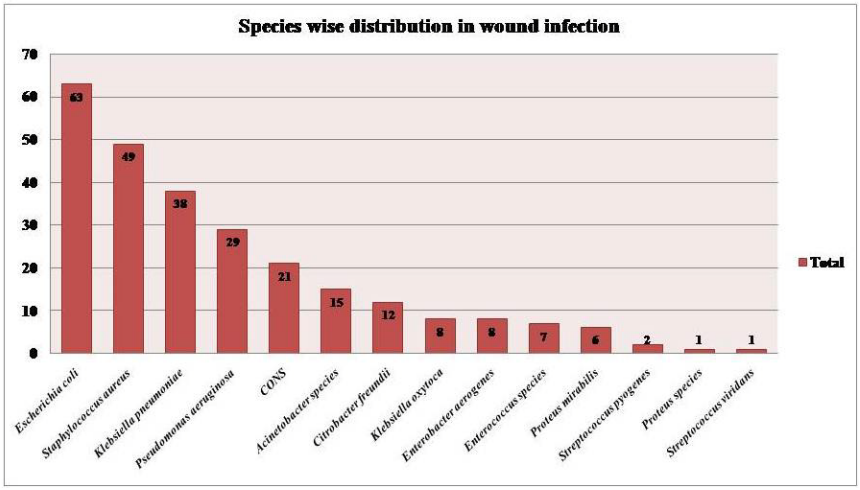

Fig. 7: Species-wise distribution of organisms causing wound infections

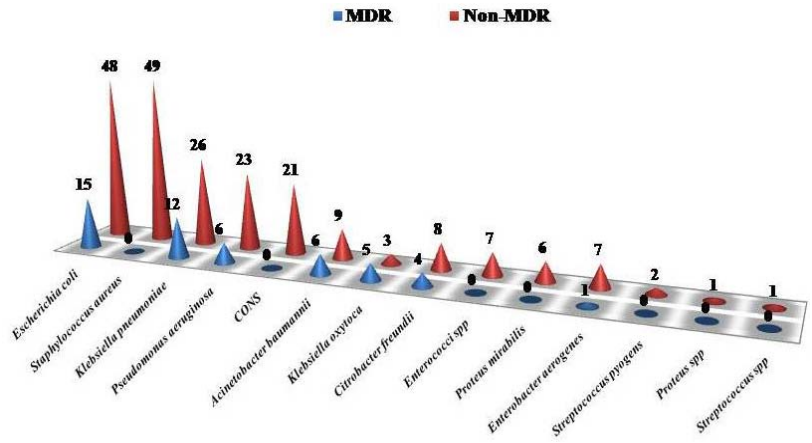

Fig. 8: Species-wise distribution of multi-drug-resistant and nonmulti-drug-resistant organisms

Table 2: Ward-wise distribution of sample

Ward	No. of samples	Percentage
Surgery ICU	108	41
Post-operative Ward	72	28
Surgery ward	53	20
Allied ward	27	11

Table 3: Species-wise distribution in wound infection

Organism	Total	Percentage
Escherichia coli	63	24
Staphylococcus aureus	49	18.8
Klebsiella pneumoniae	38	15.1
Pseudomonas aeruginosa	29	11.3
CONS	21	8
Acinetobacter species	15	5.78
Citrobacter freundii	12	4.5
Klebsiella oxytoca	8	3.04
Enterobacter aerogenes	8	3.04
Enterococcus species	7	2.6
Proteus mirabilis	6	2.3
Streptococcus pyogenes	2	0.78
Proteus species	1	0.39
Streptococcus viridans	1	0.39
Total	260	

Table 4: Species-wise distribution of multi-drug-resistant and non-multi-drug-resistant organisms

Organism	MDR	Percentage	Non-MDR	Percentage
Escherichia coli	15	24	48	76
Staphylococcus aureus	0	0	49	100
Klebsiella pneumoniae	12	31.5	26	68.5
Pseudomonas aeruginosa	6	20.6	23	79.4
CONS	0	0	21	100
Acinetobacter baumannii	6	40	9	60
Klebsiella oxytoca	5	62	3	38
Citrobacter freundii	4	33	8	67
Enterococci spp	0	0	7	100
Proteus mirabilis	0	0	6	100
Enterobacter aerogenes	1	14.3	7	85.7
Streptococcus pyogens	0	0	2	100
Proteus spp	0	0	1	100
Streptococcus spp	0	0	1	100
Total	49		211	

DISCUSSION

The detection and isolation of pathogenic organisms from various samples like wound swabs, pus or tissues by culture and sensitivity to guide the management of wound infections. Standardizing the diagnostic methods and improvising antibiotic stewardship will limit the spread of antibiotic resistance [1]. The present cross-sectional study, 14 microbial species were isolated from wound infections. The gram-negative bacteria were around 70%andthe Gram-positive bacteria were about 30%; similar results were also

reported by Guan *et al.*, Rahim K *et al.,* and Wu YK *et al.* [18-20]. In this study, most of the wound infections were caused by single bacterial species, and a similar finding was reported by Mohammed *et al.* from wound swabs with 81.7% single bacterial growth [21]. WhereasGuan *et al.* and Yeong *et al.* showed multi-drug resistant polymicrobial cultures from wound infections within 72 h, and the predominant isolate was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *Escherichia coli* [18, 22]. Glik J *et al.* mentioned *S. aureus* was predominant, followed by *P. aeruginosa* in polymicrobial wound infections [23]. Our study found that 2-3% of cultures showed two organisms. However, the combination of two organisms has been reported with *S. aureus* in association with *E. coli* 6.8% and *A. baumannii* 5.1%. Similarly, polymicrobial infection by *P. aeruginosa*is associated with *P. mirabilis*5.1%. The co-infection with three different organisms like *K. pneumoniae*, *S. aureus*, and *S. marcescens* has been observed in 3.4% of wound infections [18, 24-26]. The polymicrobial wound infections with these organisms make eradicating microorganisms more difficult. The Horizontal gene transfer between microorganisms enhances the non-healing tendency or chronic wound infections by polymicrobial organisms [24-29]. In the present study, we detected 18.8% of isolates showed multi-drug-resistance against at least five antimicrobial agents; in particular, the species involved were *E. coli, K. pneumoniae, P. aeruginosa, A. baumannii, Citrobacter species* and frequently seen isolate was methicillin-resistant Staphylococcus but not multi-drug-resistant. Co-infection is also seen among this organisms. The management of polymicrobial wound infections and its challenges are multi-drug resistance, biofilm formation, and tolerance to antimicrobials. The multi-target drugs or combination of a different group of drugs make them more bactericidal than giving higher antibiotics alone [28-32].

The multidrug-resistant *E. coli, Klebsiella species, Citrobacter, Pseudomonas aeruginosa* and *Acinetobacter baumannii* showed more resistance against ampicillin, followed by gentamicin, ceftriaxone, ceftazidime, piperacillin and ciprofloxacin [26-29, 33]. The antibiotics like amikacin, and imipenem worked well against all gram-negative multi-drug-resistant organisms in our study about 72%, and 85% respectively except a few isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa.* Similarly organisms showed sensitivity to amikacin and imipenem by Guan *et al.* 77% and 100% and Li L *et al.*70% and 83%, respectively [18, 34]. Wong *et al.* reported chronic wound infections caused by gram-negative organisms showed a good response to amikacin and meropenem [26]. Our study showed vancomycin, and cotrimoxazole are more active against gram-positive organisms; several other studies supported the same [18, 26, 33-35]. Other studies carried out by Puca V*et al.* [36] from 2017 to 2019 isolated more gram-negative organisms than gram-positive organisms; similar findings were reported in our research, including drug resistance.

CONCLUSION

The wound infections are further complicated in the presence of immunodeficiency, diabetes, and chronic vascular ulcers. Henceforth, understating the mechanism behind the wound and infections like pathogenic organisms are essential in the treatment and follow-up of patients. The microorganisms causing wound infections, increasing antimicrobial resistance towards them, and challenges in managing the same increase hospital stay and healthcare costs. This study highlights the prevalence of causative organisms and antimicrobial resistance in managing wound infections. This study will enlighten about organisms causing wound infections and the empirical therapy and switch over to correct antibiotics as soon as possible to avoid misuse of antimicrobials and to prevent the spread of drug-resistant strains among the community and hospital setup. Similar studies should in conducted in other hospital setups in a particular locality with antibiotic sensitivity, reasons for the spread of drug-resistant strains, and proper antibiotic stewardship can prevent the further spread of drug resistance.

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LIMITATIONS OF THE STUDY

The virulence factors and associated predisposition with wound infections and molecular level of characterization and drug-resistant genes detection of the different species were not able to be implemented. This study needs further extension work related proper selection of antibiotics and implementation of local antibiotic policy to prevent multi-drug resistance.

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Nil

AUTORS CONTRIBUTIONS

The Concept, the title, complete write-up, statistical analysis, photos, editing and presentation were done by Prasanna S (First and Corresponding author). The data collection, compilation of data, and pictures editing work were done by Anto PV (Second author). The Editing and compilation of details were done by Nikunja Kumar Das (Third author).

COFLICT OF INTERESTS

Nil

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