Int. J Med. Pharm. Res.
E-ISSN: 2958-3683 | **P-ISSN**: 2958-3675
Available on: https://ijmpr.in/

International Journal of
Medical and Pharmaceutical Research

ORGINAL ARTICLE OPEN ACCESS

ESKAPE Pathogens identified in wound infections and its antibiotic susceptibility pattern

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OPEN ACCESS

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Received: 07-07-2025
Accepted: 12-08-2025
Available Online: 29-08-2025

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ABSTRACT

Introduction: The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, Enterobacter*) are a group of highly virulent bacteria which are highly resistant to antibiotics and are the main cause of nosocomial infections. In recent years, there is an increase in the multidrug resistant organisms which cause pyogenic infections, so in this study we are concerned about the ESKAPE pathogens and their antibiotic susceptibility pattern.

Materials and Methods: A Retrospective cross-sectional study during the study period of January 2024 to June 2024 was undertaken. All the Pus or tissue or swab samples from wound infections were collected, processed for bacteriological identification and antibiotic sensitivity testing was done. The quantitative descriptive data analyzed in this study is mentioned as numbers and percentages. Microsoft excel sheet was used for data tabulation and for minor calculations.

Results: A total of 337 pus swabs were processed, among them 156 (46.2%) were culture positive. Out of 156 organisms, 49 (31.4%) were *Escherichia coli*, 29 (18.5%) *Staphylococcus aureus*, 46 (29.4%) *Klebsiella pneumoniae*, and 32 (20.5%) were *Pseudomonas aeruginosa*. Gram negative isolates were highly susceptible antibiotics among gram negative pathogens were amikacin (99.2%), gentamicin (88.9%), meropenem (87.4%), and levofloxacin (81.8%). 44.9% of Escherichia coli isolates, 50% of *Klebsiella pneumoniae* isolates were multidrug resistance pathogens and 18.8% of *Pseudomonas aeruginosa* isolates were MBL producers. Out of 156 organisms, 49 (31.4%) were Escherichia coli, 29 (18.5%) *Staphylococcus aureus*, 46 (29.4%) *Klebsiella pneumoniae*, and 32 (20.5%) were *Pseudomonas aeruginosa*. Gram positive were high susceptible towards cotrimoxazole (82.7%), tetracycline (82.7%), levofloxacin (75.8%), followed by moderate susceptibility to cefoxitin (58.6%), clindamycin (55.1%), cefuroxime (41.3%), erythromycin (41.3%) and penicillin (27.5%). MRSA was noted in 41.4%.

Conclusion: ESKAPE pathogens are difficult to treat, this problem will enhance if the culture and sensitivity testing is not being done at the earliest stage of infections which can lead to treatment failure and can be associated with severe complications like septicemia, acute kidney injury, prolonged hospital stay and even lead to death.

Keywords: ESKAPE pathogens, Wound infections, antibiotic.

INTRODUCTION

Pyogenic infections are collections of pus due to invasion and multiplication of microorganisms which are characterized by local inflammation of skin, soft tissues and bodily parts. Skin prevents underlying tissues from becoming colonized and invaded by the potential pathogens. Any injury that causes physical disruption of the skin integrity leads to exposure of subcutaneous tissue, resulting in an infection of the wound by external pathogens [1]. These microorganisms feed on tissues and also release toxic metabolites which destroy neutrophils forming abscesses.

Pus/pus swab is a common clinical specimen collected from individuals with several types of infections including cellulitis, impetigo, pustules, diabetic wounds, septic arthritis, osteomyelitis, soft tissue infections. Most common etiological pathogens isolated from pyogenic infections were *Staphylococcus aureus, Streptococcus, Escherichia coli,*

Klebsiella spp, Proteus spp, Pseudomonas spp. [2]. Along with pathogens the other risk factors also play a vital role in delay in the wound healing such as patient health condition involving comorbidities, age, immune status, nutrition status, severity of trauma and vascular insufficiency [3,4].

Multidrug resistant pathogens are emerging now-a-days by acquiring horizontal transfer of resistance genes or secretion of enzymes or alteration of structure or bypassing the metabolism or halting the antibiotic sequestration. Worldwide spread of these pathogens has led to increased hospital mortality and morbidity, making it a global health problem [5,6]. The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, Enterobacter*) are a group of highly virulent bacteria which are highly resistant to antibiotics and are the main cause of nosocomial infections. The acronym is sometimes extended to ESKAPEE to also include Escherichia coli [7,8].

In the United States it has been estimated that >2 million people suffer infection by some multidrug-resistant organism (MDRO) each year and about 23 000 die as a direct result of these infections [9]. In the European Union, taking data from 2007, it has been estimated that MDRO infections cause about 25,000 deaths annually and generate an overall cost of about €1500 million per year [10]. In 2050, about 10 million people a year could die worldwide if the situation does not change [11].

Many research works have been projected about multidrug resistant pathogens in wound infections. As the epidemiology and microbiota of diseases and its pathogens varies from region to region, understanding the knowledge of pathogens, antibiotics and antibiotic resistance of a community is very important for the management of a disease and also aid in infection control measures.

In recent years, there is an increase in the multidrug resistant organisms which cause pyogenic infections, so in this study we are concerned about the ESKAPE pathogens and their antibiotic susceptibility pattern. The aim of this study is to assess the impact of important ESKAPE pathogens on Wound Infections by analyzing bacteriological profiles and antibiotic sensitivity patterns of those.

MATERIAL AND METHODS

Study design & settings: A Retrospective cross-sectional study during the study period of January 2024 to June 2024 was undertaken. All the Pus or tissue or swab samples from wound infections were collected from clinical departments of Government General Hospital, Kurnool and the processing was done in the Microbiology department.

Ethics: Ethical approval was obtained from the Institutional ethics committee before conducting the study (Approval Letter No- 637/2024). Prior consent was obtained from all participants.

Sample collection: All pus samples from patients attending the OPD (outpatient department) or admitted in wards with infected wounds that were collected following standard procedures and received to the Microbiology lab were used for the study. During the period of study, around 337 pus samples from wounded sites of both diabetic and non-diabetic patients with complications like venous ulcers, superficial abscesses, and traumatic injuries were collected and included in the study. The patients belonging to both genders and age group of 01 to 90 years were included.

Inclusion criteria: The pus samples (pus aspirate and wound swab) from patients attending the outpatient department (OPD) or patients admitted in wards (in patients) with wound infections were collected in the Microbiology lab of Kurnool Medical College, Kurnool, India, between January 2024 to June 2024.

Exclusion criteria: Samples collected without following standard guidelines, pus samples collected from patients with more than one complication prescribed by the Association of Indian Medical Society, 2019.
Pus samples were collected and bacteriological identification and antibiotic sensitivity testing was done.

Quality control: All prepared biochemical and streaking media were checked for their sterility. Strains of E. coli ATCC 25922 and S. aureus ATCC 25923 were used as reference strains for quality control of AST and biochemical tests. The same strain of E. coli was also considered as a negative control during the screening and phenotypic confirmation (DDST) tests of ESBL producing Gram-negative bacilli.

Procedure: Collected samples were streaked on selective media such as 5% sheep blood agar, Mannitol Salt Agar, and MacConkey agar and incubated at 37 °C for 24h. Direct microscopic examination of Gram-stained smears of isolates. Additional tests included Coagulase test, Sorbitol fermentation, Arabinose fermentation test, other sugar fermentation test, species specific identification tests, Optochin and Bacitracin sensitivity test, and specific biochemical tests to identify Enterobacteriaceae members. After 24 hours of incubation, the bacteria were identified by colony characteristics and biochemical reactions.

Antibiotic sensitivity testing: Antimicrobial susceptibility of the isolates was assessed on Muller Hinton Agar plates using Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards institute (CLSI) guidelines. The list of antibiotics tested include amikacin (Amk-30 µg), bacitracin (Bac-10 µg), cefipime(Cef-30 µg), ceftazidime (Cez-30 µg), cefoxitin (Cex-30 µg), cefotaxim (Cet-30 µg), ceftriaxone (Ceo-30 µg),cefuroxime (Ceu-30 µg), chloramphenicol (Chl-30 µg), ciprofloxacin (Cip-5 µg), clindamycin (Cli-2 µg),colistin (Col-10 µg), erythromycin (Ery-15 µg), gentamycin (Gen-10 µg), gentamycin high (GeH-120 µg), imipenem (Imp-10 µg), levofloxacin (Lev-5 µg), linezolid (Lin-30 µg), meropenem (Mer-10 µg), penicillin (Pen-10 µg), piperacillin (Pip-10 µg), tazobactum (Taz -10 µg), polimixin B (Pol-300 µg), tetracyclin (Tet-30 µg), cotrimoxazole (1.25 µg), teicoplanin (Tei-30 µg), and vancomycin (Van-30 µg).

Data Collection: Patient details such as age, sex, occupation, comorbidities, socioeconomic status, associated symptoms and signs, and microbiological details were tabulated in an excel sheet to evaluate further.

Statistical analysis: The quantitative descriptive data analyzed in this study is mentioned as numbers and percentages. Microsoft excel sheet was used for data tabulation and for minor calculations.

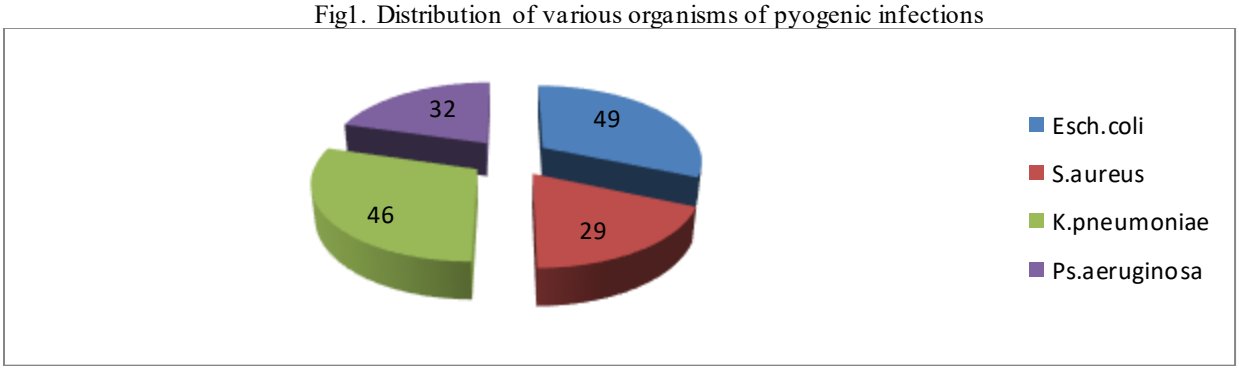
RESULTS

In this study, we are assessing pyogenic infections from the ESKAPE pathogens. Pyogenic infections were predominantly noted in male population (n=218), the male female ratio is 1.5. Out of 337 samples, 63 (18.6%) were between 15 – 28 years, 158 (46.8%) were between 29 – 55 years and 116 (34.4%) remaining were above 55 years.

A total of 337 pus swabs were processed, among them 156 (46.2%) were culture positive. Out of 156 organisms 127 (81.4%) were gram negative bacteria and remaining 29 (18.5%) were Staphylococcus aureus, which is a gram positive cocci. In this study no gram positive organisms observed other than Staphylococcus aureus.

Out of 156 organisms, 49 (31.4%) were *Escherichia coli*, 29 (18.5%) *Staphylococcus aureus*, 46 (29.4%) *Klebsiella pneumoniae*, and 32 (20.5%) were *Pseudomonas aeruginosa*.

Fig1. Distribution of various organisms of pyogenic infections



All the gram negative isolates were sensitive to colistin. Highly susceptible antibiotics among gram negative pathogens were amikacin (99.2%), gentamicin (88.9%), meropenem (87.4%), and levofloxacin (81.8%). 65% of isolates were sensitive to piperacillin+tazobactum, 70% of isolates were sensitive to cotrimoxazole and 50% of isolates were sensitive to cephalosporins and amoxyclav.

44.9% of *Escherichia coli* isolates, 50% of *Klebsiella pneumoniae* isolates were multidrug resistance pathogens and 18.8% of *Pseudomonas aeruginosa* isolates were MBL producers.

Table 1. Antibiotic susceptibility pattern of Gram negative bacteria

S.No	Antibiotics	*Escherichia coli* (n=49)	*Klebsiella pneumoniae* (n=46)	*Pseudomonas aeruginosa* (n=32)
1.	Amikacin	49 (100%)	45 (97.8%)	32 (100%)
2.	Amoxyclav	27 (55.1%)	23 (50%)	IR
3.	Cotrimoxazole	38 (77.5%)	33 (71.7%)	IR
4.	Levofloxacin	40 (81.6%)	37 (80.4%)	27 (84.3%)
5.	Ceftazidime	30 (61.2%)	29 (63.04%)	20 (62.5%)
6.	Cefoxitin	31 (63.2%)	29 (63.04%)	20 (62.5%)

7.	Piperacillin +Tazobactam	33 (67.3%)	30 (65.2%)	22 (68.7%)
8.	Cefotaxime	27 (55.1%)	23 (50%)	IR
9.	Ceftriaxone	27 (55.1%)	23 (50%)	IR
10.	Cefipime	30 (61.2%)	28 (60.8%)	19 (58.3%)
11.	Gentamicin	47 (95.9%)	40 (86.9%)	26 (81.2%)
12.	Meropenem	45 (91.8%)	40 (86.9%)	26 (81.2%)
13.	Colistin	49 (100%)	46 (100%)	32 (100%)

Based on the antibiotic susceptibility testing, all Staphylococcus aureus were 100% susceptible to vancomycin (100%), teicoplanin (100%), linezolid (100%). High susceptible pathogens were towards cotrimoxazole (82.7%), tetracycline (82.7%), levofloxacin (75.8%), followed by moderate susceptibility to cefoxitin (58.6%), clindamycin (55.1%), cefuroxime (41.3%), erythromycin (41.3%) and penicillin (27.5%). MRSA was noted in 41.4%.

Table 2. Antibiotic susceptibility pattern of *Staphylococcus aureus*

S.No	Antibiotics	Sensitivity	Resistance
1.	Penicillin	27.5% (n=8)	72.5%
2.	Erythromycin	41.3% (n=12)	58.7%
3.	Clindamycin	55.1% (n=16)	44.9%
4.	Cefoxitin	58.6% (n=17)	41.4%
5.	Cefuroxime	41.3% (n=12)	58.7%
6.	Levofloxacin	75.8% (n=22)	24.2%
7.	Tetracycline	82.7% (n=24)	17.3%
8.	Cotrimoxazole	82.7% (n=24)	17.3%
9.	Teicoplanin	100%	-
10.	Vancomycin	100%	-
11.	Linezolid	100%	-

DISCUSSION

Pyogenic infections are due to wide variety of organisms and are characterized by typical inflammatory response. This entity is easy to detect by clinical examination but it is difficult to predict a pathogen behind this inflammation, this can lead to treatment failures and is associated with severe complications, such as cellulitis, necrotizing fasciitis, septicaemia, and the patient can finally goes into multiorgan dysfunction failure and death. Pyogenic infections can be prevented by knowing the microbiotia of the community and the antibiogram of the hospital. For many years it is a common health problem and the patients are being facing the recurrence of infections, so it is the time to change and raise awareness about the knowing the pathogen and choosing the accurate antibiotic to manage infections.

Most of the pyogenic infections are treatable if it is diagnosed and investigated in the early stage of the disease. Patients requiring treatment should be treated with the accurate antibiotics. Isolation of multidrug resistant pathogens in such patients needs to be thoroughly managed and education to family members is required regarding infection control practices. The microbiotia pattern also changing which is a multifactorial so there is a definite need to go for microbiological culture.

A total of 337 pus swabs were processed in this study, among them 156 (46.2%) were culture positive. Rijap BP et al [12] did a study in Nepal they noted 65% of bacterial culture positivity in pyogenic infections. Upreti N et al [13] observed 62% of overall bacterial growth rate. Growth rate depends on multiple factors such as chronicity of the disease, usage of antibiotics, comorbidities, hygiene measures and many more. The faster the diagnosis the more yield can be observed in the specimens.

Pyogenic infections were predominantly noted in male population (n=218), the male female ratio is 1.5. Out of 337 samples, 63 (18.6%) were between 15 – 28 years, 158 (46.8%) were between 29 – 55 years and 116 (34.4%) remaining were above 55 years. Out of 156 organisms, 49 (31.4%) were *Escherichia coli*, 29 (18.5%) *Staphylococcus aureus*, 46

(29.4%) *Klebsiella pneumoniae*, and 32 (20.5%) were *Pseudomonas aeruginosa* as observed in this study. Pyogenic infections in relation to multidrug resistance bacteria study was conducted in western Rajasthan, India [14]. They observed male predominance with the male to female ratio is 1.6:1. Most common age group was 21-40 years. Most of their specimens were from wound which showed *Staphylococcus aureus* (30.9%) followed by *Escherichia coli* (24.76%), *Pseudomonas aeruginosa* (16.68%), *Klebsiella* (14.4%). Hassan MA et al [15] documented Gram-negative bacteria showed dominance with a ratio of 63.6%, while Gram-positive bacteria reported 36.4%. A study from Nepal [12] observed gram positive pathogens as predominant bacteria representing as 57.4%. *Staphylococcus aureus* (412, 49.28%), *Escherichia coli* (136, 16.27%), *Klebsiella spp*. (88, 10.53%), and *Pseudomonas spp.* (44, 5.26%) were the common pathogens isolated. In contrast a multidrug resistant wound infections study in Nepal [13] noted most number of pyogenic cases in younger age group of ≤10 years it was 82.1%. They also observed gram positive predominance in similar to another Nepal study by Rijal BP et al [12]. Upreti N et al [13] studied that among 116 bacterial isolates, *Staphylococcus aureus* was the most predominant bacteria (56.9%) followed by *Escherichia coli* (8.6%), *coagulase negative staphylococci* (7.8%), *Acinetobacter spp.* (5.2%), *Klebsiella pneumoniae* (5.2%), *Pseudomonas aeruginosa* (4.3%), *Enterococcus spp.* (4.3%), *Citrobacter freundii* (2.6%), *Proteus vulgaris* (1.6%) and *P. mirabilis* (0.9%).

As per this study gram negative isolates were sensitive to colistin. Highly susceptible antibiotics among gram negative pathogens were amikacin (99.2%), gentamicin (88.9%), meropenem (87.4%), and levofloxacin (81.8%). 65% of isolates were sensitive to piperacillin+tazobactum, 70% of isolates were sensitive to cotrimoxazole and 50% of isolates were sensitive to cephalosporins and amoxyclav. All *Staphylococcus aureus* were 100% susceptible to vancomycin (100%), teicoplanin (100%), linezolid (100%). High susceptible pathogens were towards cotrimoxazole (82.7%), tetracycline (82.7%), levofloxacin (75.8%). Kalita JM et al noted [14] most of the Gram negative isolates showed high resistance towards cephalosporin, cotrimoxazole and quinolones and Gram positive cocci showed high resistance towards penicillin and quinolone group of drugs. Vancomycin resistant *Enterococci* were surprisingly noted very high which was 16.98%. Rijal BP et al [12] concluded that Gram positive isolates were resistant to ampicillin, ciprofloxacin, cotrimoxazole, erythromycin, and cloxacillin. Gram negative isolates were resistant to cephalosporins but were well susceptible to amikacin and imipenem. Upreti N et al [13] did a study on pyogenic infections that mentioned as both Gram positive (73.3%) and negative (78.8%) isolates showed high frequency of sensitivity to gentamicin. Ahmed EF et al [16] from Upper Egypt did a study on wound infections they clearly stated piperacillin, methicillin, ampicillin/sulbactam, and amoxicillin/clavulanic acid were all highly resistant to S. aureus and Coagulase-negative Staphylococci. The prevalence of methicillin-resistant S. aureus in wound infections was 89.9%. S. aureus showed superior sensitivity to vancomycin (85.3%) and linezolid (81.3%). The highest prevalence of Gram-negative isolates was for *Pseudomonas aeruginosa* (40%), which was highly sensitive to ciprofloxacin (79.2%) and highly resistant to levofloxacin (83.3%). An NV et al [17] by Vietnam research workers highlighted that the drug ampicillin displayed the highest resistance (91.9%), while colistin and ertapenem remained the most effective. In Gram-positive bacteria, glycopeptides like teicoplanin and vancomycin (0% and 3.3% resistance, respectively) were most effective. Clindamycin and tetracycline showed decreasing effectiveness.

Rasmi AH et al [18] did an intensive study on Staphylococcus isolates from wound infections. MRSA isolates accounted for 91.5%. The multidrug resistance (MDR) percentage in S. aureus isolates was 54.2%. They noted high sensitivity pattern against vancomycin, linezolid, and chloramphenicol. However, a high resistance pattern was observed against oxacillin and piperacillin. sea was the most predominant gene (72.9%), followed by icaA (49.2%), hla (37.3%), and fnbA (13.6%). sea was the commonest virulence gene among MRSA isolates (72.2%), and a significant difference in the distribution of icaA was found. However, sea and icaA were the commonest genes among MSSA isolates (79.9%). The highest distribution of sea was found among ciprofloxacin-resistant isolates (95.2%).

ESKAPE pathogens in this study noted as 44.9% of *Escherichia coli* isolates, 50% of *Klebsiella pneumoniae* isolates were multidrug resistance pathogens and 18.8% of *Pseudomonas aeruginosa* isolates were MBL producers. MRSA was noted in 41.4%. Kaljita JM et al [14] noted 74.79% of *Klebsiella* and 74.32% of *Acinetobacter species* were MDR bugs. MRSA was observed in 13.26% isolates and Inducible clindamycin resistance among Staphylococcus aureus isolates was observed in 16.19%. Upreti N et al [13] stated among *S. aureus* isolates, 60.6% were MRSA strains, whereas 40% of *K. pneumoniae* and 33.3% of *C. freundii* were ESBL producing bacteria followed by *E. coli* (25%). Ahmed EF et al [16] did a MDR pathogens study revealed several isolates had multi-drug resistance profile (52.4%). The overall MDR rate of Gram-positive and Gram-negative isolates were 50% and 54.9%, respectively. An NV et al [18] concluded Gram-negative bacteria exhibited a 63.6% MDR rate. *Acinetobacter baumannii* showed the highest MDR rate (88.0%).

MDR bacteria hinder the healing process of wounds and also reduce the cell migration and tissue regeneration by producing toxins and forming biofilms. They are resistant to multiple antibiotics leading to start broad spectrum or reserved antibiotics, which inturn, causes higher side effects and economic loss to patients [19]. Wound healing process is more delayed in MDR bacteria when compared to non MDR bacteria, this increases the risk of complications like tissue necrosis and infections [20]. Comorbidities like diabetes, compromised immune systems, or those undergoing extensive surgery are particularly vulnerable to MDR infections and associated wound healing complications [21].

CONCLUSION

From this study we conclude that male predominance in wound infections and it is quite common in middle aged persons. Pyogenic infections are caused by both gram positive and gram negative organisms. Gram negative were high susceptible to carbapenems, beta lactam and beta lactamase inhibitors, aminoglycosides and broad spectrum antibiotics. Gram positive isolates were sensitive to vancomycin, linezolid, teicoplanin, but their use is limited. 41.1% of isolates were MRSA. Multidrug resistant pathogens percentage is almost half of the culture isolates. ESKAPE pathogens are difficult to treat, this problem will enhance if the culture and sensitivity testing is not being done at the earliest stage of infections which can lead to treatment failure and can be associated with severe complications like septicemia, acute kidney injury, prolonged hospital stay and even lead to death.

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