



RESEARCH ARTICLE

A RETROSPECTIVE STUDY ON THE OCCURRENCE OF ENTEROBACTERIACEAE AS ETIOLOGICAL AGENTS OF PYOGENIC INFECTIONS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Members of the family Enterobacteriaceae are important causative agents of pyogenic infections in both hospitalized patients and community-acquired infections. Objectives: The objective of the study was to determine the occurrence of Enterobacteriaceae causing pyogenic infections, assess their mono- or poly-microbial etiology, and evaluate the antimicrobial susceptibility patterns of the isolates. **Methods:** Clinical samples received for aerobic bacterial culture were subjected to Gram staining and cultured on appropriate media. Isolates were identified using standard microbiological techniques and antimicrobial susceptibility testing was performed according to CLSI guidelines. **Results:** Among 122 Enterobacteriaceae-positive culture samples, 94 (77%) were mono-microbial and 28 (23%) were poly-microbial. Sensitivity pattern showing tigecycline (56%), netilmicin (48%) and cotrimoxazole (47%) sensitive which was followed by cefoperazone + sulbactam (42%), gentamicin (34%), meropenem (57%), ceftriaxone (20%), ciprofloxacin (19%), cefepime (19%), ampicillin+sulbactam (16%), piperacillin+ tazobactam (16%), nalidixic acid (13%), aztreonam (13%), amikacin (11%), cefexime (11%), ceftazidime (9%), cefuroxime (6%), amoxicillin + clavulanate (3%). **Conclusion:** The occurrence of pyogenic infections caused by Enterobacteriaceae varies across geographical regions and hospital settings, highlighting the need for periodic surveillance to guide appropriate antimicrobial therapy.

INTRODUCTION

Pyogenic infections are an important cause of wound sepsis and are commonly associated with trauma, burns, and surgical procedures¹. These infections are characterized by the production of pus resulting from local inflammation caused by the invasion and multiplication of pathogenic microorganisms². Pyogenic infections of wounds are among the leading causes of morbidity and mortality worldwide which may further proceed to sepsis if proper treatment is not initiated³. Suppurative wound infections have been reported to vary between 3% and 11% in developed countries and are estimated to be as high as 40% in developing countries. Pyogenic infections are significant infectious diseases encountered in hospitals worldwide. These are associated with prolonged hospitalization, increased morbidity and are a huge economic burden to the country⁴. Pyogenic bacteria may be either from gram negative organisms especially belonging to order Enterobacterales as well as gram positive, aerobes or facultative anaerobes⁵. A large number of Methicillin resistant Staphylococcus aureus (MRSA) along with multidrug resistant gram-negative isolates are increasing

as important cause of pyogenic infections in recent years⁶. The routine use of antibiotics has resulted in wide spread antibiotic resistance especially within the gram-negative organisms⁷. Antibiotic resistance is seen as potential danger around the world and infection caused by such bacteria increases morbidity and mortality, resulting in significant economic loss due to the usage of more expensive antibiotics to treat these infections⁸.

MATERIAL AND METHODS

The current study was conducted in the Department of Microbiology, Goa Medical College, Bambolim, Goa for a period of 6 months from October 2024 to March 2025. The clinical specimens included wound swabs, pus, slough, aural swabs, tissue samples, and body fluids obtained from patients with pyogenic infections. A total of 455 clinical samples were received for aerobic culture from outpatient and admitted patients with pyogenic infections. All samples were subjected to primary gram staining for Pus cells and bacteria; following which, they were cultured on Blood agar, MacConkey agar and Glucose broth. All inoculated media were incubated at 37°C

for 24 hours. Growth on culture media was identified by standard microbiological techniques. This included colony morphology, hemolysis on Blood agar, gram staining, motility and biochemical reaction. Some strains were confirmed by Automated Identification System using VITEK2 Biomerieux. Antimicrobial susceptibility testing was done for all isolates by Kirby Bauer disc diffusion techniques as per according to CLSI M100, 34th edition (2024)¹². Samples yielding mixed flora with 3 or more organisms were excluded from the study.

RESULTS

A total of 455 clinical samples were processed during the study period. Enterobacterales were isolated from 122 (26.8%) samples. From each sample type the total number of samples received and the number of culture positive samples amongst them were as follows:

Table 1. Distribution of Enterobacteriaceae-positive samples according to specimen type

| Sample type | Number of Samples | Positive samples |
|--------------|-------------------|------------------|
| Wound swab | 170(37.3%) | 46(37.7%) |
| Pus | 132(29%) | 32(26.2%) |
| Slough | 72(15.8%) | 21(17.2%) |
| Fluid | 36(7.9%) | 9(7.4%) |
| Vaginal swab | 27(5.9%) | 7(5.8%) |
| Tissue | 13(2.9%) | 5(4.1%) |
| Aural Swab | 5(1.1%) | 2(1.6%) |
| Total | 455 | 122(26.8%) |

The mono-microbial (77%) and poly-microbial (23%) distribution of culture positive samples with organisms belonging to *Enterobacteriaceae* family were as follows:

Table 2. Distribution of Enterobacteriaceae-positive samples according to organism type

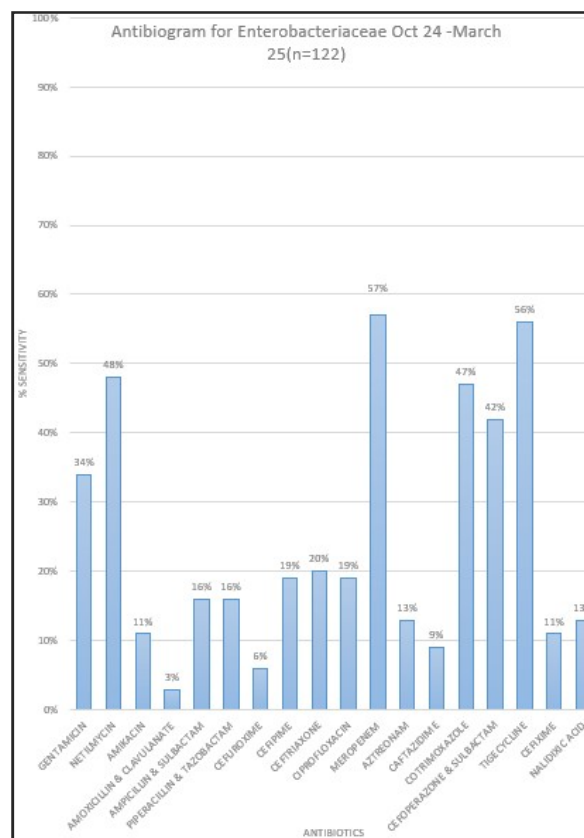
| Organisms | Mono-microbial | Poly-microbial | Total culture positive samples |
|-----------------------------|----------------|----------------|--------------------------------|
| <i>Klebsiella species</i> | 49(52.1%) | 9(32.1%) | 58(47.5%) |
| <i>Escherichia coli</i> | 17(18.1%) | 7(25%) | 24(19.6%) |
| <i>Enterobacter species</i> | 10(10.6%) | 5(18%) | 15(12.3%) |
| <i>Proteus mirabilis</i> | 5(5.3%) | 2(7.1%) | 7(5.7%) |
| <i>Proteus vulgaris</i> | 4(4.3%) | 2(7.1%) | 6(5%) |
| <i>Citrobacter diversus</i> | 6(6.4%) | 1(3.6%) | 7(5.7%) |
| <i>Citrobacter freundii</i> | 3(3.2%) | 2(7.1%) | 5(4.1%) |
| Total | 94(77%) | 28(23%) | 122 |

Samples with poly-microbial growth of *Enterobacteriaceae* with other secondary microorganisms was as mentioned below:

Table 3. Distribution of Enterobacteriaceae-positive samples as per poly-microbial etiology

| Primary Organisms | Secondary Organisms |
|-----------------------------|---|
| <i>Escherichia coli</i> | <i>Pseudomonas species</i> (3) <i>Klebsiella species</i> (1) <i>Enterobacter species</i> (1) <i>Citrobacter species</i> (1) <i>Enterococcus species</i> (1) |
| <i>Klebsiella species</i> | <i>Pseudomonas species</i> (4) <i>Citrobacter species</i> (3) <i>Proteus species</i> (1) <i>MSSA</i> (1) <i>MRSA</i> (1) <i>CoNS</i> (1) |
| <i>Enterobacter species</i> | <i>Pseudomonas species</i> (4) <i>CoNS</i> (1) |
| <i>Proteus mirabilis</i> | <i>Enterococcus species</i> (1) |
| <i>Citrobacter species</i> | <i>Pseudomonas species</i> (4) |

The antibiotic susceptibility pattern of the above isolates was as shown in the graph below.



DISCUSSION

Pyogenic infections are significant type of infection frequently encountered in hospital globally¹. Wound infections can increase the length of hospital stay and accounts for the mortality rate up to 70-80%³. The etiological agents include both Gram-positive and Gram-negative pathogens, with Gram-negative organisms predominating⁴. Moreover, continuous upsurge of AMR is a therapeutic challenge⁶. Therefore, updated knowledge of etiology and Antibiogram is highly valuable to reduce morbidities⁶. The predominant organisms isolated were *Klebsiella species* (47.5%), followed by *Escherichia coli* (19.6%). This is comparable to a study showing *Klebsiella species* (31 %) including *Klebsiella pneumoniae* and *Klebsiella oxytoca* followed by *Escherichia coli* (25.7%) conducted in Gurugram, Haryana 2023 study by Devi, et al¹. Similarly in study conducted in Nagpur MH 2023 Bajare et al² reported *Klebsiella species* (27%) followed by *Escherichia coli* (20.2%). Other Enterobacteriaceae isolated included *Enterobacter species* (12.3%), *Proteus mirabilis* (5.7%), *Citrobacter diversus* (5.7%), *Proteus vulgaris* (5%), *Citrobacter freundii* (4.1%). About 23 % culture positive samples were poly-bacterial having associated infections with other *Enterobacteriaceae* family organisms or with *Pseudomonas aeruginosa*, coagulase negative staphylococcus species (CoNS) and *Enterococcus species*. This pattern is similar to study conducted in Shahajahanpur, UP 2023 Diwakar et al⁸. The pattern of organisms is also similar to study conducted in Mewat, Haryana 2016 Sangwan et al⁹. In our study, *Enterobacteriaceae* showed highest sensitivity to tigecycline (56%), netilmicin (48%) and cotrimoxazole (47%). This was followed by cefoperazone- sulbactam (42%), gentamicin (34%), meropenem (57%), ceftriaxone (20%),

ciprofloxacin (19%), cefepime (19%), ampicillin-sulbactam (16%), piperacillin+ tazobactam (16%), nalidixic acid (13%), aztreonam (13%), amikacin (11%), cefexime (11%), ceftazidime (9%), cefuroxime (6%), amoxicillin-clavulanate (3%) indicating a high burden of antimicrobial resistance when compared with previous study in Chamrajanagar, Karnataka 2020 by Wadekar et al⁷ showing piperacillin + tazobactam (88.7%) and meropenem (85.4%) sensitivity. A study conducted in Shahajahanpur, UP 2023 Diwakar et al⁸ shows maximum sensitivity to imipenem comparable to our study showing maximum sensitivity to meropenem (57%).

CONCLUSION

This study highlights that the distribution of Enterobacteriaceae causing pyogenic infections varies across geographical regions and healthcare settings. Regular surveillance of bacterial etiology and antimicrobial susceptibility patterns is essential for effective empirical therapy and antimicrobial stewardship. These infections are mainly caused by *Klebsiella* species, *Escherichia coli*, *Enterobacter* species, *Proteus mirabilis*, *Citrobacter diversus*, *Proteus vulgaris* and *Citrobacter freundii*. Very few of them are having associated infections with *Pseudomonas aeruginosa*, coagulase negative *staphylococcus* species (CoNS) and *Enterococcus* species. As antimicrobial susceptibility pattern varies with time and geographical area, more studies with molecular level analysis should be conducted at intervals.

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