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IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ENTEROBACTER SPECIES BY VITEK-2 SYSTEM ISOLATED FROM VARIOUS CLINICAL SPECIMEN IN GOVT MEDICAL COLLEGE KOTA (RAJASTHAN)

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ABSTRACT **Background:** Enterobacter were proposed as a genus in 1960 by Hormaeche and Edwards based on the division of the former genus Aerobacter into motile, ornithine decarboxylase (ODC)–positive strains (Enterobacter) and nonmotile ODC-negative strains (Klebsiella). The Vitek-2 system is the second generation of Vitek and offers a more sophisticated model of data analysis as well as a fully automated process for card identification, organism suspension dilution and card filling. **Aim and Objectives:** To study identification and antimicrobial susceptibility pattern of Enterobacter species by Vitek-2 system isolated from various clinical samples. **Material and Methods:** A total of 100 Enterobacter species obtained from various clinical samples like urine, pus, sputum, endotracheal aspirate and body fluids (pleural, ascitic, peritoneal and CSF) etc. of patients received at Department of Microbiology, Government Medical College & Associated Group of Hospitals, Kota during a period of approximately 1 year from May 2021 to May 2022 were taken for the identification and Antibiotic sensitivity testing by Vitek-2 system. **Result:** Out of 100 Enterobacter isolates, 69% were E.cloacae and 31% were E.aerogenes. Antimicrobial susceptibility results of Enterobacter species revealed the susceptibility of 56.41% for Nitrofurantoin, 69% for Piperacillin/ Tazobactam and 72% for Cefoperazone/ salbactam. **Conclusion:** Enterobacter seems to be emerged with increasing resistance to multiple antibiotics.

KEYWORDS : Vitek-2 system, Antimicrobial susceptibility, E.cloacae, E.aerogenes.

INTRODUCTION

Enterobacter were proposed as a genus in 1960 by Hormaeche and Edwards based on the division of the former genus Aerobacter into motile, ornithine decarboxylase (ODC)–positive strains (Enterobacter) and nonmotile ODC-negative strains (Klebsiella). Enterobacter species are considered opportunistic pathogens, causing disease mainly in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation.[1] The species E.cloacae and E.aerogenes are the main pathogens in the genus which are associated most commonly with lower respiratory tract infection (LRTI) and urinary tract infection (UTI) followed by bacteremia, skin and soft tissue infections, intra abdominal infections, endocarditis, osteomyelitis, septic arthritis, CNS and ophthalmic infections.[2]

Enterobacter species are members of the ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species), which are described as the leading cause of resistant nosocomial infections.[3,4,5,6–13] These bacteria possess inducible chromosomally encoded beta-lactamases and through plasmid mediated resistance, are becoming resistance to many classes of antibiotics including third generation cephalosporins and carbapenems.[14,15,16]

Treatment of infections with Enterobacter spp. is notoriously difficult and broad resistance to third generation cephalosporins, penicillin and quinolones is an increasing problem.[17] Rapid bacterial identification and susceptibility testing improve patient therapy and outcome, decreases emergence of resistance.[18,19] There is a need to provide rapid, efficient and accurate system for identification and antimicrobial susceptibility testing of pathogens. In this regard the automated identification/AST systems aid in rapid diagnosis/treatment of bacterial pathogens.[20]

The Vitek-2 system is the second generation of Vitek and offers a more sophisticated model of data analysis as well as a fully automated process for card identification, organism suspension dilution and card filling.[21] Vitek-2 is an automated system utilizing growth based technology used for identification and antimicrobial susceptibility testing of bacteria and yeast. It is an integrated modular system that consists of a filling-sealer unit, a reader- incubator, a computer control module, a data terminal and a multicopy printer. These system accommodate the colorimetric (for identification) and turbidometric (for AST) reagent cards that are incubated and interpreted automatically.[22,23]

This study was aimed to determine the identification and antimicrobial susceptibility pattern of Enterobacter species isolated from various clinical samples. Common species of Enterobacter causing various infections was also identified. This would provide important information regarding the empirical therapy of Enterobacter infections and also reduce treatment failure in hospitalized patients.

MATERIAL AND METHOD

A total of 100 Enterobacter species obtained from various clinical samples like urine, pus, sputum, endotracheal aspirate and body fluids (pleural, ascitic, peritoneal and CSF) etc. of patients received at Department of Microbiology, Government Medical College & Associated Group of Hospitals, Kota during a period of approximately 1 year from May 2022 to May 2023 were taken for the identification and Antibiotic sensitivity testing by Vitek-2 system.

Identification and antimicrobial susceptibility testing by Vitek-2 system was confirmed on the basis of results obtained by performing following steps;

- A sterile swab or applicator stick was used to transfer a sufficient number of colonies of a microorganism and to suspended the microorganism in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube.
- The turbidity was adjusted to 0.50-0.63 Mcfarland and measured using a calibrated turbidity meter called the DensiChek™. This suspension was used for identification.
- After preparing microorganism suspension, fixed volume of 145 microlitre (Gram negative bacilli) of suspension preparation was transferred into a another clear plastic test tube containing 3.0 ml of sterile saline for AST.
- Identification and AST cards were inoculated with microorganism suspensions using an integrated vacuum apparatus.
- Inoculated cards were passed by a mechanism, which cut off the transfer tube and were sealed the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards.
- All card types were incubated on-line at 35.5 + 1.0ºC. Each card was removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time.
- During incubation, each test reaction was read every 15 minutes to measured either turbidity or colored products of substrate metabolism.

RESULTS

Figure 1 showed the distribution of isolated Enterobacter into different species. Out of 100 Enterobacter isolates, 69% were E.cloacae and 31% were E.aerogenes. About 68% were isolated from IPD and 32% from OPD.

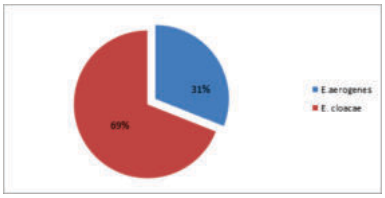

Figure1: Distribution Of Enterobacter Isolates Into Species

Table No. 1: Specimen Wise Distribution

Specimen	NO. of isolates	Percentage
Urine	78	78%
sputum	10	10%
Pus	6	6%
Tracheal aspirates	3	3%
Pleural fluid	3	3%
Total	100	100%

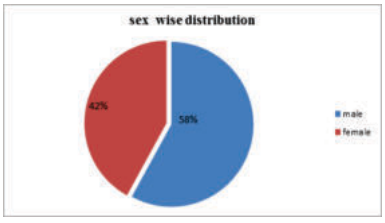

Figure 2: Sex Wise Distribution Of Enterobacter Isolates

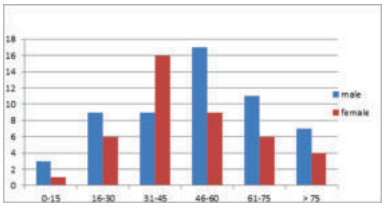

Figure 3: Age And Sex Wise Distribution Of Enterobacter Isolates

Table 2: Antimicrobial Susceptibility Pattern By Vitek-2 System Among Enterobacter Isolates

Antimicrobial agents (No =100)	Susceptible(S) No. (%)	Intermediate(I) No. (%)	Resistant(R) No. (%)
Nitrofurantoin (No =78)	44 (56.41%)	0	34 (43.58%)
Piperacillin/ Tazobactam	69 (69%)	-	31 (31%)
Cefoperazone/ salbactam	72 (72%)	0	28 (28%)
Ceftriaxone	35 (35%)	0	65 (65%)
Gentamicin	67 (67%)	0	33 (33%)
Amikacin	71 (71%)	0	29 (29%)
Ciprofloxacin	51 (51%)	2 (2%)	47 (47%)
Norfloxacin (No =78)	63 (80.76%)	0	15 (19.23%)
Imipenem	88 (88%)	0	12 (12%)
Cefixime	28 (28%)	0	72 (72%)
Co -trimoxazole	66 (66%)	2 (2%)	32 (32%)
Tetracycline	49 (49%)	0	51(51%)

DISCUSSION

Enterobacter strain commonly arise from the endogenous intestinal flora of hospitalized patients but can occur in common source outbreaks or are spread from patient to patient. Infections are especially common in patients who have received antimicrobial therapy and in those in intensive care units. The Enterobacter species exhibit intrinsic resistance to a wide range of antibiotics. It also shows acquired resistance to 3rd generation cephalosporin. In recent years, Enterobacter has turned out to be an important agent of nosocomial infections.

In this study, Out of 100 Enterobacter isolates, E.cloacae 69% and E.aerogenes 31% were most common. This study is similar to studies done by Monteiro et al[24] in which E.cloacae 61.90% and E.aerogenes 38.09% were most common.

In this study, Enterobacter species were more commonly isolated form inpatients 68% than outpatients 32%. This is comparable with the study of Nida OZCAN et al[25] and Kumar V.et al[26] where IPD patients were more 72% and 78% than OPD patients 28% and 22% followed by study of Yaz1c1 et al[27] where IPD patients were more 65% than OPD patients 35%.

In present study, Out of 100 Enterobacter isolates, 78% were isolated from urine, 10% from sputum, 6% from pus, 3% from tracheal aspirates and 3% from pleural fluid. This is comparable with the study of Mashrura Quraishi et al[28], Nida OZCAN et al[25] and Kumar V. et al[26] where 68%, 46.6% and 23% were isolated from urine, 2%, 2% and 17% from sputum, 11.8%, 8% and 36% from pus respectively.

In present study, Enterobacter species were isolated from males 58% and female 42.%, which is comparable with the study of Nida OZCAN et al[25], Sujatha Bhat et al[29] and Kumar V.et al[26] where 51.7%, 57.35% and 76% were isolated from male and 48.3%, 42.64% and 24% from female.

In this study, Out of 100 Enterobacter isolates, 04% were between the age group of 0-15 years, 15% were between the age group of 16 to 30 years, 25% were between the age group of 31 to 45 years, 26% were between the age group of 46-60 years, 19% were between the age group of 61-75 years and 11% between the age group of >75 years which is comparable with the study of Sujatha Bhat et al[29] where 14.70% were between the age group of 1-9 years, 4.41% were between the age group of 10-19 years, 20.58% were between the age group of 20-29 years, 13.23% were between the age group of 30-39 years, 7.35% were between the age group of 40-49 years and 39.70% were between the age group of 50-59& >60 years.

In present study, Enterobacter isolates were susceptible to 56.41% for Nitrofurantoin, 69% for Piperacillin / Tazobactam, 72% for Cefoperazone / salbactam, 35% for Ceftriaxone, 67% for Gentamicin, 71% for Amikacin, 51% for Ciprofloxacin, 80.76% for Norfloxacin, 88% for Imipenem, 28% for Cefixime, 66% for Co-trimoxazole and 49% Tetracycline which is comparable to the study of Sujatha Bhat et al[29] where Enterobacter isolates were 78.94% susceptible to Gentamicin, Amikacin (91.22%), Norfloxacin (92.98%), Imipenem (98%) and Co-trimoxazole (73.68%). Karlow sky et al[30] also reported susceptibility of Amikacin (96.1%) and Imipenem (93.5%) for Enterobacter isolates. Mansour et al[31] also reported susceptibility of Gentamicin (95.7%), Imipenem (96.1%) and Co-trimoxazole (66.7%) by Vitek -2 system.

CONCLUSION

Enterobacter seems to be emerged with increasing resistance to multiple antibiotics. Extended survey should be launched in larger hospitals of our country to determine the true prevalence of Enterobacter causing nosocomial infections. Regular monitoring of antimicrobial resistance of Enterobacter should be done and Infection control program for prevention of nosocomial infection should be practiced in all the hospitals of our country.

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