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Research Article

BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF LACTOSE-FERMENTING GRAM-NEGATIVE BACILLI IN VARIOUS CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Members belonging to the family Enterobacteriaceae are the most frequently encountered bacterial isolates recovered from clinical samples. They have emerged as an impending pathogenic entity with the ability to show resistance for commonly used antimicrobials. The present study was undertaken to detect the clinical distribution and antibiogram profile of lactose fermenting Gram-negative bacilli [LFGNB] isolated from various specimens. **Materials and Methods:** This study has been conducted in the department of Microbiology at a tertiary care teaching hospital from July 2015 to December 2016. A total of 415 LFGNB isolated from various clinical specimens were identified and antibiotic sensitivity test was performed by subjecting them to VITEK -2 compact system. **Results:** Urine was the most commonest specimen followed by blood culture. Culture positivity was highest in urine samples (43%) followed by blood culture (30%). E coli was the predominant isolate (58%) followed by Klebsiella species (36%). Antibiotic susceptibility testing showed that majority of the isolates were sensitive only to Colistin (93.49%), Tigecycline (81.93%) and Ertopenem (72%) with decreased susceptibility and resistance to other groups of drugs, thus revealing multidrug resistance. **Conclusion:** E.coli and Klebsiella species are the predominant organisms of nosocomial infections in our hospital. It is necessary to identify them and to monitor their susceptibility pattern to guide the clinician for better care and management of patients. Hence, antibiotic sensitivity testing and infection control measures are needed to prevent the spread of multidrug resistant LFGNB in health care settings.

INTRODUCTION

Gram negative bacilli belonging to the family Enterobacteriaceae are the most frequently encerintered bacterial isolates recovered from clinical specimens[1]. They are ubiquitously present, reported worldwide and popular members of aerobic bacterial flora of human intestine. They are common causative agents of a variety of nosocomial and community acquired infections like UTI's, septicaemia, pyogenic infections etc[2]. Currently drug resistance to these pathogenic bacteria is frequently being reported worldwide.

In India, the reasons for development of antimicrobial resistance could be due to irrational use of antibiotics, over the counter availability of higher or broad antimicrobial agents, higher prevalence of infection and poor monitoring of antibiotic susceptibility in hospitals[3]. Extensive use of broad spectrum antibiotics in hospitalised patients has led to the development of multidrug resistant strains, which are associated with increased morbidity and mortality[4].

Hence, this study was undertaken to isolate and identify lactose fermenting Gram-negative bacilli [LFGNB] from various clinical specimens and to determine the antibiotic susceptibility pattern at a tertiary care teaching hospital.

METHODS AND MATERIALS

A total of 415 strains which grew in culture from various clinical specimens -Urine, Blood culture, Tracheal aspirates, Sputum, Pus and Body fluids, isolated at the clinical Microbiology Laboratory, NRIGH during the period from July 2015 to December 2016 were included in the study. Only LFGNB that grew well in MacConkey agar were included.

Inoculum Preparation

From the isolated colonies grown on the media, a bacterial suspension was prepared in 3 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12x75 mm clear plastic (polystyrene) test tube. The turbidity of the suspension was adjusted to a McFarland standard of 0.5 with the help of a

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VITEK-2 DensiCheck instrument. The time between the preparation of inoculum and filling of the card was always less than 30 min.

Identification with the VITEK-2 compact system was performed using a Gram Negative (GN) card according to the Manufacturer's instructions. The 64 well plastic GN card contains 41 tests including 18 tests for sugar assimilation, 18 tests for sugar fermentation, 2 decarboxylase tests and 3 miscellaneous tests (for urease, utilization of malonate and tryptophan deaminase). The culture suspension was inoculated into the GN card with the help of a vacuum device inside the filling chamber. The cards were later transferred into the loading chamber where the cards were sealed and were incubated in a rotating carousel at 37^0C. Each loaded card was removed from the carousel for every 15 minutes, transported to the optical system for reaction readings and the returned to the carousel incubator until the next read time. Data was collected at 15-minute intervals during the entire incubation period.

Quality control

The Vitek-2 compact machine was validated using the standard strains as per the manufacturer's instructions. E.coli ATCC25922, K. pneumoniae ATCC 700603 and Enterobacterhormaechei ATCC 700323 were used. During the study period, the control strains were checked at regular intervals.

Antimicrobial susceptibility testing

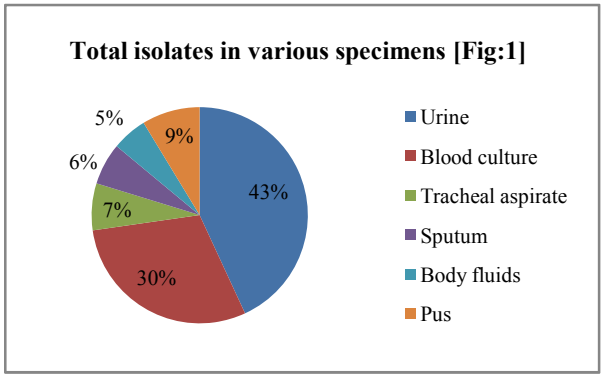
Antimicrobial Susceptibility testing with the VITEK-2 compact system was performed using an AST N281 card according to the Manufacturer's instructions. The VITEK-2 AST N281 susceptibility card is intended for use with the VITEK-2 systems in clinical laboratories as an *in-vitro* test to determine the susceptibility of clinically significant aerobic gram negative bacilli to antimicrobial agents. A panel of twenty five antibiotics were tested in AST N281 card.

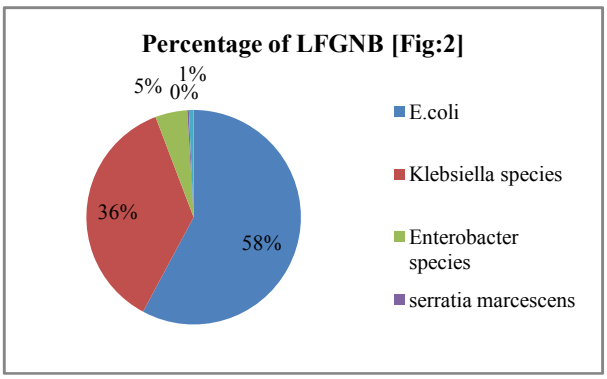
The cards were filled with an inoculum (Prepared by transferring 200µL of culture suspension from the 0.5 McFarland culture suspension used for filling the identification cards into a fresh 3mL sterile saline solution obtaining a final turbidity of 8x106 cfu/mL) in the filling chamber. The VITEK-2 System automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The VITEK-2 compact system subsequently corrects, where necessary for MIC's or clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations.

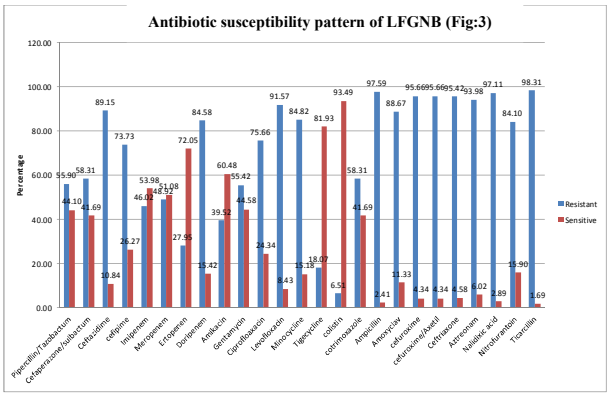
RESULTS AND DISCUSSION

A total of 415 strains collected from various clinical specimens were subjected to VITEK – 2 compact system. Out of 415, strains 179 strains were isolated from urine, 123 from blood cultures, 36 from pus/wound infections, 29 from tracheal aspirates, 26 from sputum, and 22 from various body fluids [ascitic fluid, peritoneal fluid etc] [Fig:1].So, culture positivity was highest in urine samples [43%] followed by blood cultures [30%]. This is in accordance with other studies[5,6].

In our study all the LFGNB isolated from various clinical specimens belonged to the family Enterobacteriaceae [Fig:2]. Members belonging to Enterobacteriaceae may account for 80% of clinical significant isolates of Gram-negative bacilli. They account for nearly 50% of septicemia cases, more than 70% of UTI, a significant percentage of intestinal infections. They are the leading causes of nosocomial infections [7].







Of the total 415 LFGNB isolated, E.coli was the predominant isolate in urine 137 [76.5%], blood culture 59 (48%), body fluids 16 (72.7%) and pus 18 (50%), followed by Klesbsiella species [Urine 39 (21.7%), blood culture 56 (45.5%) pus 13 (36%) and body fluids 5(22.7%)]. Highest yield of E.coli was observed in urinary specimens which was in accordance with other studies [5,6] . E.coli is the most common cause of UTI's in humans and leading cause of enteric infections and systemic infections [8].

Culture positivity for K pneumoniae which was the predominant species in respiratory specimens was Tracheal aspirates 75.8%, sputum 61.5 % and that of blood was 45.5% which was quite higher than those reported in other Indian studies, reporting 30.9% and 28.4% followed by blood (45.5%) [9,10]. K.pneumoniae can be considered as the major cause of

different types of lower respiratory infections followed by septicaemia. However, the isolation rate of K. pneumoniae from pus (36%) and urine (21.7%) was low which can be compared with other references stating 21.1% and 26.6% respectively [11,12].

Observations in the study indicate that both E.coli and K pneumoniae can be stated as the major causes of different types of infections and as a potent nosocomial pathogens in our hospital. Even though the isolation rates of Citrobacter and Enterobacter species were low in this study, studies have shown that they are emerging as significant pathogens too[13].

Antibiotic sensitivity testing revealed that most of the isolates were susceptible to colistin(93.49%), Tigecycline (81.93%) and Erotpenem (72 %). On the contrary, low level of susceptibility was found to Meropenem (51.08%), Imipenem (53.9%) and high level of resistance was exhibited against third generation cephalosporins, cefipime, Ampicillin, Amoxyclav, cefuroxime Axetil and Nalidixic acid [Fig:3]. The decreased susceptibility of cephalosporins could be due to the production of ESBL and Ampc Beta-lactamases. Studies from Chandigarh (87% - 89%) and Nigeria (84.8% - 96%) too have reported high level of resistance to cephalosporins[14,15].

Among the fluoroquinolones tested the isolates showed high resistance to ciprofloxacin (75.6 %) and Levofloxacin (84.8 %). High resistance to these drugs have been reported in other studies too, stating 63% as high as 76.9% strains to be resistant [14,16]. Sensitivity to piperacillin/Tazobactum and cefaperazone/ sulbactum was 40% - 45 %.

From this study it is clear that majority of the isolates were multidrug resistant. They were showing high resistance to the commonly used antibiotics and high sensitivity to those drugs which were to be kept reserved for more serious conditions of the patient.

Antibiotic overuse, prescription of drugs with lack of proper sensitivity test and over dosing may have created this problem. Multidrug resistance and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease course [17]. Because antimicrobial resistance patterns are continuously evolving and multidrug resistance organisms undergo progressive antimicrobial resistance, continuously updated date on antimicrobial susceptibility profiles is essential to ensure the provision of safe and effective empiric therapies [18].

CONCLUSION

The present study highlighted the fact that LFGNB, E.coli and K.pneumoniae have emerged as potential nosocomial pathogens in our hospital. It highlights the most alarming situation of highly diverse antibiotics resistance. Regular surveillance of antibiotic susceptibility pattern may help to overcome the indiscriminate use of antibiotics, a major cause of emergence of drug resistance among pathogens. The data of this study may be used to determine trends in antimicrobial susceptibilities to formulate local antibiotic policies and overall to assist clinicians in the rational choice of antibiotic therapy.

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