

ISOLATION AND CHARACTERIZATION OF LACTOSE AND NON-LACTOSE FERMENTING BACTERIA FROM TERTIARY CARE HOSPITAL AND THEIR ANTIMICROBIAL SUSCEPTIBILITY TEST

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ABSTRACT

Objective: The purpose of the present study was to identify the fermenting and non-fermenting Gram-negative bacteria from the tertiary care hospital.

Methods: The conventional method of identification by biochemical analysis and antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method. Furthermore, analysis of microbes was done by Vitek-2.

Results: 424 strains of lactose fermenting Gram-negative bacilli (LFGNB) and non-LFGNB (NLFGNB) were isolated from 3097 clinical samples. From the total LF bacteria, *Escherichia coli* was the predominant isolate accounting for 50.94% specimens, followed by *Klebsiella pneumoniae* 27.59% and *Enterobacter* 0.47%. From the total NLFGNB, *Acinetobacter baumannii* was the predominant isolate accounting for 12.73% specimens followed by *Pseudomonas aeruginosa* 6.13%, other isolates were *Stenotrophomonas maltophilia* 1.17%, *Burkholderia cepacia* 0.94%. In the present study, male were more infected than female. The study also showed that LF bacteria were more infectious than non LF bacteria and isolates were from urine samples.

Conclusion: Both NLFGNB and LFGNB Gram-negative bacilli were found to be major contaminants, and are important pathogenic bacteria causing a wide range of infections in the tertiary care hospital.

Keywords: Lactose fermenting Gram-negative bacteria, Vitek-2, Tertiary care hospital, Kirby-Bauer disc diffusion, Lactose non-fermenting Gram-negative bacteria.

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INTRODUCTION

The emergence of antibiotic resistance is due to excessive and often unsupervised use of antibiotics in humans and animals. Risk factors for the spread of resistant bacteria in hospitals and the community are due to lapse in hygiene or poor infection control practices. Increasing antibiotic resistance in bacteria has been aggravated by the slow pace in developing newer antibiotics. The present study focused mainly on Enterobacteriaceae which are lactose fermenters (LFs) and some non-lactose such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The purpose of clinical microbiology is to isolate and identify pathogenic microorganism and to assist in the diagnosis, management, and treatment of infectious diseases. Immunocompromised patients are highly susceptible to environmental strain which aggravate with invasive techniques such as catheterization, bronchoscopy, colposcopy, or surgical biopsies [1].

These organisms can occur frequently as components of commensal flora of man and animals and are regular contaminants of the hospital environment. These pathogens have been implicated in various ranges of infections. These bacteria are emerging opportunistic organisms causing a wide variety of nosocomial infections such as infectious wounds, ventilators associated pneumonia, blood stream infection, intensive care unit infection, and urinary tract infection (UTI) [2].

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE) and multi-drug resistant Gram-negative bacteria can spread by direct or indirect person-to-person contact. Independent risk factors for MRSA include the use of broad spectrum antibiotics, the presence of decubitus ulcers and prosthetic

devices while those for VRE include prolonged hospitalization and treatment with glycopeptides or broad spectrum antibiotics. For the spread of resistant Gram-negative bacteria, risk factors include urinary catheterization, excessive use of antibiotics, contaminants of humidifiers and nebulizers.

The spread of penicillin-resistant pneumococci (PRP) and drug-resistant/multidrug-resistant tuberculosis (MDRTb) is due to airborne transmission. Risk factors for the spread of PRP include overcrowding, tracheostomies and excessive use of penicillins for viral respiratory infections; for MDRTb they include poor compliance, the convergence of immunosuppressed patients, delayed diagnosis or treatment, and poor or inadequate ventilation and isolation facilities [3].

The main LF organism includes *Escherichia coli*, *Klebsiella pneumoniae*, and non-LF organisms include *A. baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia*. In hospital environment, these non-fermenting Gram-negative bacilli affect seriously ill patients by interfering with the treatment and may also lead to septicemia. The cause of infection could be the hospital environment, i.e., mainly device related infections which are often resistant to disinfectants. Devices have the potential to spread the infection from patient-to-patient via fomites or hands of medical personnel's along with that they spread from soap cakes for hand washing in hospitals or through dust on the baby weighing machines [4].

Extended spectrum beta-lactamase (ESBLs) are enzymes conferring broad resistance to penicillin, cephalosporin, and monobactam but not to carbapenem [5]. ESBLs are often plasmid mediated, and most are

members of TEM-1, TMH-2 and SHV-1 family of enzymes. These enzymes are produced by *Enterobacteriaceae* mainly by *E. coli*, *K. pneumoniae*, and *Klebsiella oxytoca*. They have been detected in other Gram-negative bacilli such as *Salmonella* species, *Proteus* spp., *P. aeruginosa*, and other *Enterobacteriaceae* [6]. Resistant to beta-lactamase is most commonly associated with the production of high levels of naturally produced cephalosporinase [7]. Carbapenem resistance is primarily caused by two mechanisms, either by reducing intracellular concentration or by hydrolysis of the drug [8]. Penicillin binding proteins all may contribute to carbapenem resistance.

Developing a resistance to antibiotics is natural to microbes, which cannot primarily be a cause of constantly involving nature of microbes to chemical around them. There are different mechanisms for resistance in Gram-negative bacilli as production of enzymes, enzymatic inactivation of antimicrobial agents, alternations in target sites, loss of outer membrane proteins, spontaneous mutation, or by DNA transfer [9]. The phenomenon is very important regarding its partial and economic implications.

The patients who receive long courses of broad-spectrum antibiotics, corticosteroids, antimetabolites or anticancer drugs or undergo complete surgical procedures and AIDS patients are more prone to be infected by these pathogens which are resistant to multiple drugs. These microorganisms have survived for thousands of years by their ability to adapt to antimicrobial agents [10].

METHODS

Source of sample collection

Totally, 3097 samples from the clinically suspected cases were collected from a tertiary care health setup. The samples were received in the microbiology laboratory such as blood, tissue, pus; respiratory samples were - Endotracheal secretion, bronchoalveolar lavage (BAL), sputum. Aseptic conditions were strictly maintained throughout the examinations.

Isolation of bacterial isolates and identification

Samples were cultured according to the sample type as urine sample plated on CLED agar, and respiratory samples were plated on blood agar, MacConkey agar, and chocolate agar. Other samples were plated on blood agar, MacConkey and brain heart infusion broth such as pus and tissue. Blood samples were plated on blood, and MacConkey agar and smears were prepared of all the samples for Gram-staining except urine samples. They were allowed to incubate at 37°C for 24 hrs. The bacterial isolates were observed for colony morphology, hemolytic pattern, and gram's reaction. Biochemical tests were performed for identification of bacterial strains as per the Standard Conventional Method [11].

Antibiotic susceptibility testing

Antibiotic susceptibility test of bacterial isolates was performed according to the criteria of Clinical and Laboratory Standards Institute [12] using the Kirby-Bauer disc diffusion on Muller-Hinton agar (MHA). Isolates were grown in peptone water at 37°C turbidity was matched with 0.5 McFarland standards [13]. The lawn culture was done on MHA plate, and antibiotics disc were placed. Plates were incubated at 37°C overnight and zone of inhibition was observed according to CLSI guidelines [14]. Plates were observed after 24 hrs as inhibition zone diameter sensitive, resistant, and intermediate ESBL positive isolates were isolated. ESBL isolates were tested with ceftazidime and ceftazidime-clavulanic acid on MHA agar.

Vitek-2

Instrument based on colorimetric measurements for the identification as well as antibiotic susceptibility cards were used for higher accuracies.

Modified Hodge test (MHT)

All the carbapenem resistant strains were subjected to MHT for detection of carbapenemases. The suspension of ATCC *E. coli* 25922

was prepared in comparison to 0.5 McFarland standard in 5 ml of sterile saline using the direct colony suspension. A 4.5 ml of sterile saline was pipetted out into a sterile tube. Then, 0.5 ml of the ATCC *E. coli* 25922 suspension was added to 4.5 ml of saline to make a 1:10 dilution. Diluted ATCC *E. coli* 25922 was inoculated to an MH plate containing 70 µg/mL of zinc sulfate and then streaked over the entire plate with the help of sterile cotton swab. A 10 µg meropenem susceptibility disk was placed in the center of the MH plate. Then the test organism, positive control (*K. pneumoniae* ATCC BAA-1706) and negative control (*K. pneumoniae* ATCC BAA-1705), was streaked in a straight line from the edge of the meropenem disk to the edge of the plate and then incubated at 35±2°C in ambient air for 16-20 hrs. Carbapenemase production was detected by the appearance of the enhanced ATCC *E. coli* 25922 growth along the test organism that revealed a clover-leaf-like indentation which indicated a positive test.

RESULTS

A total of 424 strains of LF Gram-negative bacilli (LFGNB) and non-LFGNB (NLFGNB) were isolated from 3097 clinical samples accounting for an isolation rate of 13.69% out of the total samples. The clinical specimen included urine 45.51%, blood 24.76%, ET 8.72%, sputum 3.77%, and BAL 2.12%, Pus 15.09% (Figs. 1 and 2).

From the total LFGNB, *E. coli* was the predominant isolate accounting 50.94% specimens followed by *K. pneumoniae* 27.59%; other isolate was *Enterobacter* 0.47% (Fig. 3). The total NLFGNB *A. baumannii* was the predominant isolate accounting 12.73% specimens followed by *P. aeruginosa* 6.13% other isolates were *Stenotrophomonas maltophilia* 1.17%, *Burkholderia Lepacia* 0.94% (Fig. 4).

Out of the total patients, 53.77% of the patients accounted for males and 46.22% were female (Fig. 5).

The isolates were found to be maximum when the duration of stay of the patient was 0-10 days, i.e., 39.62% patient followed by duration of stay of more than 30 days, i.e., 29.71% patients were infected with LFGNB and NLFGNB. While when the duration of stay was 10-20 days 23.11% were suffering from the infection and when the duration was 20-30 days 7.54% of the infection (Fig. 6). The patients aged between 40 and 75 years were more infected, i.e., 62.26% while age group of 30-45 years account 17.92% patient of age group 15-30 were 7.54%. Patient with age group of 75-80 years 5.42% patient with age group of 0-10 were 3.77 and >80 age group were 3.06% patients (Fig. 7).

A significant decrease of antibiotic susceptibility was seen. Different antibiotics used were tested which included amikacin (AK), gentamicin (CN), cefuroxime (CXM), cefixime (CFM), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), sulbactam/cefoperazone (SCF), ciprofloxacin (CIP), colistin, levofloxacin (LEV), moxifloxacin (MOX),

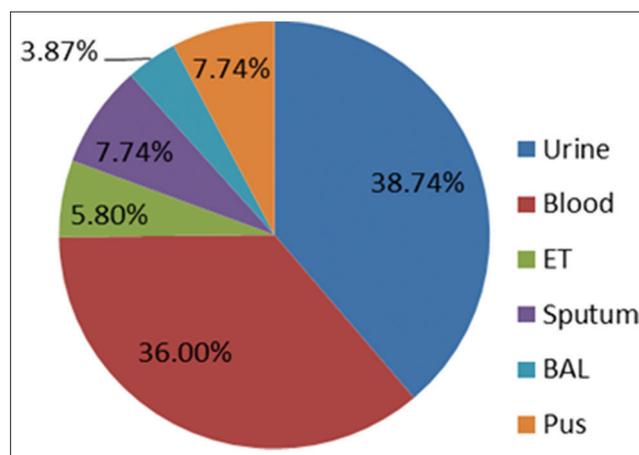


Fig. 1: Total clinical isolates

meropenem (MEM), imipenem (IPM), colistin sulphate (CT), and piperacillin/tazobactam (TZP). The most active antibiotics against LF and non-LF was colistin accounting almost 98% followed by amikacin (60%) and cefoperazone (50%) [15]. In some specimens, carbapenems (imepenem and meropenem) showed susceptibility while in most of the cases it was found to be resistant. A few specimens were multidrug resistant. Carbapenem resistance was observed in 58.9% *A. baumannii* out of 112 clinical isolates by Vitek-2 and Kirby-Bauer disk diffusion method [16]. Therefore, the carbapenem resistant isolates were subjected for carbapenemase production by MHT and 83.3% were carbapenemase producers.

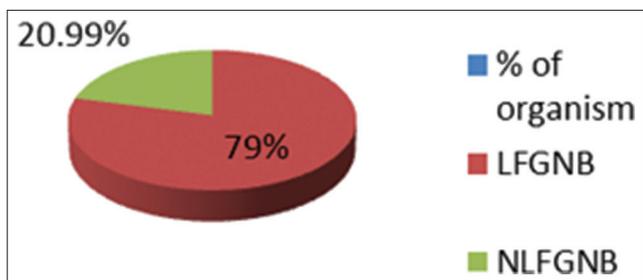


Fig. 2: Percentage of lactose fermenting Gram-negative bacilli (LFGNB) and non NLFGNB of the total isolates

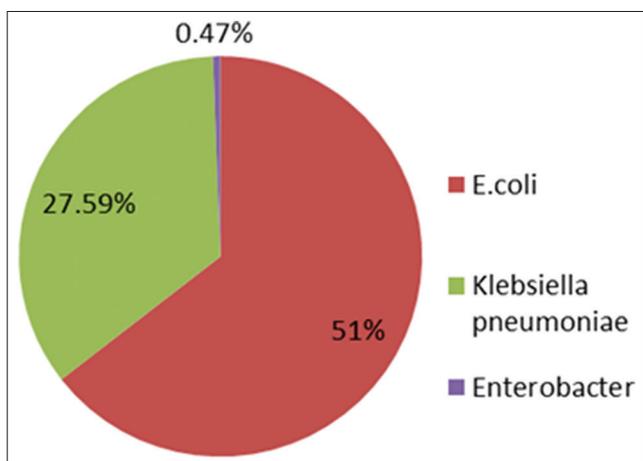


Fig. 3: Percentage of micro-organisms amongst lactose fermenting Gram-negative bacilli

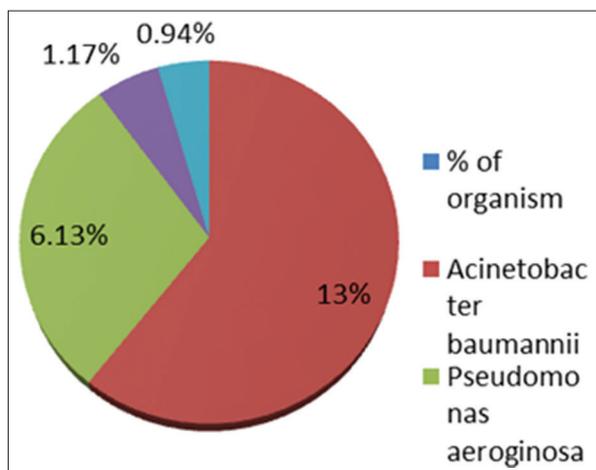


Fig. 4: Percentage of the different micro-organisms among non-lactose fermenting Gram-negative bacilli

Isolation of ESBL

It was observed that of a total of 335 isolates of Enterobacteriaceae strains, 218 were ESBL positive 65.07% over a period of 2 months detected with the help of VITEK 2 DensiChek instrument and manual method in MHA plates [17].

The plate was divided into two parts. These were again tested with ceftazidime and ceftazidime-clavulanic acid discs on one side, and E-test of cefotaxime and cefotaxime-clavulanic acid was placed on the other half. After overnight incubation, the plates were observed. An increase in the zone diameter ≥ 5 mm between the antibiotic alone and in combination was considered as ESBL positive. This is the screening test used for the detection of ESBL producer. Further, E-test (cefotaxime on one end, cefotaxime/clavulanic acid on other end) was performed on total isolates and compared. The strains with ratio of cefotaxime minimal inhibitory concentration (MIC) and cefotaxime-clavulanic acid MIC ≥ 8 indicated the presence of ESBL.

DISCUSSION

In the present study, LFs 335 (79%) were predominant, compared to non-LFs 89 (20.99%) where the majority of LF were isolated from

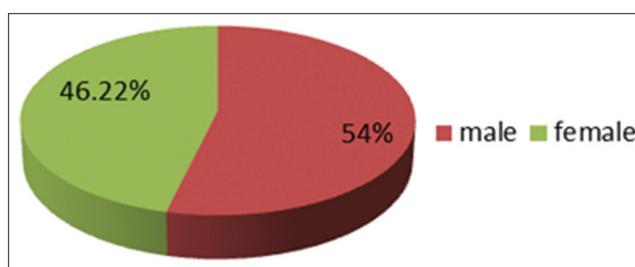


Fig. 5: Percentage of infected males and females

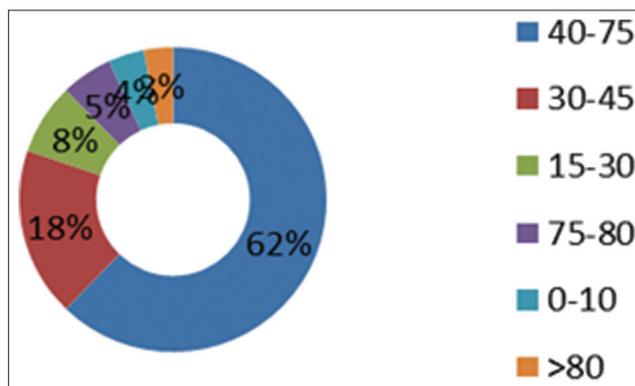


Fig. 6: Percentage of patients of different age groups

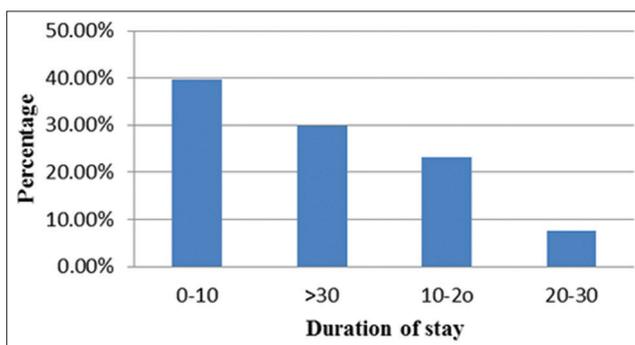


Fig. 7: Percentage of infection as per duration of stay



Fig. 8: Quadrant streaking of both non-lactose fermenting Gram-negative bacilli (LFGNB) and LFGNB along with antibiotic susceptibility test

urine samples. Enterobacteriaceae were among the leading causes of nosocomial infection. The most common isolate was *E. coli*, followed by *K. pneumoniae* in our study. Non-fermenting Gram-negative bacilli were considered to be a contaminant in the past, however, it has been observed that emergence of the strains has shown as an important health-care pathogen [18].

Similarly from a study from the All India Institute of Medical Sciences, New Delhi, in 2002 showed that 68% of the Enterobacteriaceae were ESBL producing strains [19].

The unusually high prevalence of ESBL producers in the present study was due to the overcrowded large tertiary care referral center, and the majority of infections were nosocomially acquired [20].

In a study from Kolar, Karnataka showed saprophytic nature of non-fermenting Gram-negative bacilli as the potent pathogen that exhibits resistant not only to beta-lactam and other antibiotics but also to carbapenems [21]. The sensitivity test was performed by Kirby-Bauer disc diffusion methods.

In our study, the most common non-fermenting Gram-negative bacilli were *P. aeruginosa* (53.8%) followed by *A. baumannii* (22.2%) and others (11.4%). Similarly, from a study from Delhi has reported that 172 samples were identified as non-fermenting Gram-negative bacilli out of 1526 respiratory samples. *A. baumannii* was the predominant isolate accounting 59.88% specimens followed by *P. aeruginosa* 33.13% other isolates were *S. maltophilia* 5.23% and *Burkholderia cepacia* 1.74% from the total samples. *A. baumannii* and *P. aeruginosa* were the most common non-fermenting Gram-negative bacilli isolated in the study of patients concerning respiratory tract infection was reported [14].

P. aeruginosa was the most prevalent pathogen amongst the Gram-negative bacilli in our study. *P. aeruginosa* was found to be sensitive to imipenem (94.2%) cefoperazone (70.5%), amikacin (69%) and ticarcillin (63%). Whereas *A. baumannii* was found to be 100% sensitive to imipenem and 70% to penicillin and 35% to amikacin. *S. maltophilia* was 100% sensitive to cotrimoxazole and ciprofloxacin (Fig. 8). All the carbapenem resistant strains were subjected to MHT for carbapenemase production, wherein 83.3% strains were found to be carbapenemase producers while a study from South India reported that 71% of isolates were carbapenemase producers by MHT [22].

From the study conducted in Uttar Pradesh reported that *E. coli* was highly sensitive to nitrofurantoin [23].

Furthermore, from a study by Daza *et al.* had shown that nitrofurantoin should be used as antibiotic if *E. coli* is identified. Nitrofurantoin can be an appropriate agent as the first treatment of UTI in the India population [24].

Similarly in the present study that *E. coli* was 100% sensitive to nitrofurantoin, imipenem, and netilmicin, and was highly resistant to ampicillin, while *Klebsiella* species had shown high sensitivity to

imipenem and resistance to ampicillin and amoxicillin/clavulanic acid. The effective drugs for *P. aeruginosa* were gentamycin, ceftazidime, piperacillin/tazobactam, nitrofurantoin, norfloxacin, polymyxin-B, and colistin.

In the present study, it has been found that out of the total admitted patients 53.77% of the patients accounted for males and 46.22% were females. Male patients have been found more infected than female. It showed that males are more susceptible to the infection caused by LFGNB and NLFGB. Similar trends have also been reported by a study of Eastern India, where 55% patients were males and 45% were females [25]. While study from Rotak has revealed that males and females were equally susceptible to the infection [21].

We have also observed that the longer the duration of patient's stay at the hospital more is the susceptibility to LFGNB and NLFGB.

CONCLUSION

We the authors would like to conclude that the contamination in overcrowded tertiary care hospitals is more as compared to the well sanitized and ventilated hospitals. Infection was more predominant in males as compared to females, longer the duration of stay higher is the chance to get infected. Dominant LFGNB was found to be *E. coli*. However, non-fermenting Gram-negative bacilli were *P. aeruginosa* and *A. baumannii*.

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