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Research Article

ISOLATION AND IDENTIFICATION OF GRAM -NEGATIVE BACILLI FROM CLINICAL SPECIMEN

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ABSTRACT

Introduction: Gram-Negative Bacilli (GNB) includes numerous organisms but the ones which are known to cause nosocomial infections are *Pseudomonas aeruginosa*, *Acinetobacter baumanii*. This study was undertaken to identify various Gram negative bacteria isolated from patients admitted at a hospital, in Meerut, India. **Material and Method**: A total of 3000 clinical specimens were analyzed. These included 1512(50.4%) urine, 28 (0.9%,) pus, 260(8.6%) blood, 159 (5.3%) Sputum, 45(1.5%) CSF and 38 (1.2%) body fluid samples. These samples were inoculated on blood agar, and MacConkey agar. The plates were then incubated at 37°C for 18-24 hours. The clinical isolates obtained were identified using the conventional biochemical tests as per the standardized protocols. **Result**: Majority (1366/3000) of the isolates obtained were Gram-negative bacilli clinical accounting for an isolation rate of 45.5%. Among these GNB, *Escherichia coli* was the most common isolate, accounting for 375 (27.4%) of the total, followed by *Klebsiella pneumoniae* 292(21.3%), *Acinetobacter species* 64(12%), *Enterobacter species* 139 (10%), and *Proteus* Species 134(9.8%). **Conclusion**: GNB are emerging as important opportunistic pathogens and are resistant to most commonly used antimicrobials. Therefore early diagnosis and institution of empirical therapy based on local antibiogram of the institute would reduce mortality and improve patient management.

INTRODUCTION

Gram negative bacteria (GNB) are the most common causative agent for morbidities and mortalities in patient admitted in hospitals. Gram-negative bacteria are bacteria that do not retain the crystal violet stain used in the Gram staining method for bacterial differentiation.[1] Gram negative bacteria are characterized by their cell envelopes, which is composed of a thin peptidoglycan cell wall sandwiched between an inner cytoplasmic cell membrane and a bacterial outer membrane. Gram-negative bacteria are found in virtually all environments on Earth that support life.

GNB have great clinical importance in hospitals because they put patients admitted in the intensive care units (ICU) at high risk and lead to high morbidity and mortality[2][3]. These bacteria have a great ability to cause disease in humans and can reach almost all systems in the organism, such as the digestive system, nervous system, urinary system, and Blood stream, causing diseases ranging from diarrheal gastroenteritis to severe meningitis. GNB colonize the intestines, airways, and skin, which favors the spread of these microorganisms to other parts of the human body, especially in immune compromised individuals.

One of the greatest difficulties of health professionals is to treat nosocomial infections of the lower respiratory tract in which the GNB are involved because they are responsible for a good portion of these infections and are non-responsive to antibiotic therapy due to the high resistance rates and the poor penetration of drugs into the lung parenchyma [4]. The aim of this study was to isolate and identify various bacteria isolated from clinical samples obtained from patients admitted in a hospital located in Meerut city.

MATERIAL AND METHOD

This study was carried out at western Diagnostic laboratory Meerut from June 2016- July 2021. A total 3000 sample were collected from patients admitted in a hospital in Meerut. The samples included Ascitic fluid 14(0.46%), Blood 260 (8.6%), Pus 794 (26 %), CSF 45 (1.5%), Semen 49 (1.6%), Sputum 159 (5.3%), Pleural Fluid 38 (1.2%), and Urine 1512(50.4%). All the samples were collected at the facility and transported to the laboratory. The samples were immediately inoculated into

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both differential and enriched media (MacConkey agar and blood agar plates) and then incubate at 370c for 48 hour. Identification of growth obtained was done based on colony characteristics of the organisms such as beta hemolysis on blood agar, non-lactose fermentation and pigment production (greenish yellow and bluish green pigments) on MacConkey agar. The isolates which showed these characteristics then sub cultured onto blood agar to obtain a pure culture and further characterization using the standard biochemical tests.

Gram Staining and Microscopy

Gram staining was performed on colonies from subcultures for the identification of their gram reaction. Specimens were smeared onto clean grease free glass slides and air dried and then heat fixed and Gram stained. The stained slides were examined microscopically under oil immersion lens for bacterial morphology.

Bacterial Identification

Biochemical tests included oxidase test, citrate utilization test and indole test was performed to test the enzymatic activities of the organisms. Identification was performed with the Vitek 2 compact (BioMérieux Inc. USA) [5] system using GN ID REF21341 (identification-Gram-negative bacteria) cards. All the test procedures were followed according to the manufacturer's instructions.

RESULT

Total of 3000 sample was collected from suspected patients during the study period. Of these 1536 (51%) samples were from the male patients and 1464 (49%) were from the female patients. Age of the study population ranged from 1 year to 95 years.

Of the 3000 samples inoculated, Gram negative bacilli were isolated from 1366 (45.5%) samples and Gram positive from 280 (9.3%) samples. 1354 (45%) samples were found to be sterile and no growth was obtained on the medium. The percentage of gram negative isolate in various clinical samples is given in table 1.

Table 1 Number of isolates, clinical specimen wise; n (%)

Specimen	Total gram-negative bacilli	Percentage (%)
Ascitic Fuid	2	0.14
Bile	5	0.36
Bialatery drain L	2	0.14
Bialatery drain R	2	0.14
Blood	42	3
Cornia	3	0.21
Drain	1	0.07
Drain2	1	0.07
ET Tube	6	0.43
FLUID	4	0.29
Folycil catheter	2	0.14
Fungal	1	0.07
Hvs	4	0.43
Pus	286	20.9
Semen	7	0.51
Swab	5	0.36
Sputum	71	5.1
Urine	891	65.2
Pleural Fluids	3	0.21
Centerline Tip	11	0.80
Throat	2	0.14
Tissue	8	0.58
Tracheal aspirate	7	0.51
Total	1366	

Out of total 1366 Gram negative bacilli, majority of the specimen n=375 (27.4%) were identified as *Escherichia coli*, 292(21.3%) were identified as *Klebsiella pneumoniae*, 164 (12%) were identified as *Acinetobactor Speices* , 139 (10%) were identified as *Enterobacter Species*, 134 (9.8%) were *proteus* Species, 132 (9.6%) were *Citrobacter* species, 69 (5%) were *Serratia marcescens* and 61 (4.4%) were identified as *Psedomonas aeruginosa*.

DISCUSSION

In most resource limited settings the emergence and spread of gram negative pathogens is one of the major challenges for the provision of good quality health service in hospitals. Successful management of patients with different kinds of infectious diseases depends on the isolation and identification of the bacterial pathogens. It is well articulated that Gram-negatives are predominantly isolated in different clinical specimens. They are an important cause of nosocomial infections (sepsis, pneumonia, and meningitis) and generally cause severe disease [6]

In the present study, the overall proportion of Gram negative bacilli was 45.5%. Which is similar (45.9%) to the study of Shailpreet Sidhu *et al*.; [7], Ashish Bajaj *et al*.; [8]. Other studies have also reported Gram-negative bacteria as the most common cause of BSIs. [9,10]

In this study Escherichia coli, (27.4%) was the most predominant isolate followed by *Klebsiella pneumoniae* (21.3%). The results of this study are in line with the previous. [11].

In our study 12% of the isolates were of Acinetobacter spp, 9.8% were *Enterobacter Species*, 9.6% were *Proteus Species* and 5% were *Citrobacter species.* Similar results have been reported in previous studies from India [12, 13].

In the present study, majority of the clinical isolates were recovered from, urine, pus, Pleural fluid, Swab, sputum, etc. Specifically, among the urine culture isolates, *E. coli* and *K. pneumoniae* were the major etiologic agents. This finding is in agreement with the other studies [14] [15] [16].

The frequent use of invasive devices in the form of peripheral venous catheter, urinary catheter, central venous catheter and Endotracheal Tube were found in most of the patients. These devices probably could have acted as a source of infection in these patients [17].

CONCLUSION

It can be concluded from this study that the most commonly isolated GNB are *Escherichia coli*, and *K. pneumoniae*. GNB are important causative agent of the nosocomial infections and are resistant to commonly used antibiotics. More importantly, these organisms have great potential to survive in hospital environment, so effective methods of sterilization and infection control measures should be implemented to reduce mortality and improve patient management.

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