

Identification of Different Bacterial Species and Their Antibacterial Susceptibility Tests among the Patients Admitted for Several Gastrointestinal Disorders

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Abstract:

Aim and Objective: Gastroenteritis is the most common acquired infection nowadays. The gut microbiota is the source of most strategic pathogens which have the capacity to cause most deadly infections in critically ill patients and also healthy humans. The aim of the current study was to elucidate the antimicrobial sensitivity patterns in patients admitted for different GI related diseases.

Materials and Methods: The study was carried out March 2022 to December 2023 in the Department of Gastroenterology, IMS and SUM Hospital, Bhubaneswar by targeting the hospital acquired and locality acquired infectants as bacteria and their resistance to antibiotics. The bacteria were isolated from tissue samples collected during the endoscopy procedure from Department of Gastroenterology.

Results: From 95 tissue samples, 58 showed significant growth. There were about 10 different types of bacteria were identified in the current microbial cultural process. A fraction of 21% *Klebsiella pneumoniae* followed by *Acinetobacter baumannii* (17%), *Proteus vulgaris* (15%), *Pseudomonas aeruginosa* (14%), *Enterococcus aerogenes* (12%), *Proteus mirabilis* (7%), *Klebsiella oxytoca* (7%), *Citrobacter freundii* (3%), *Escherichia coli* (2%), and *Serratiamarcescens* (2%) were isolated during the microbial isolation process. The gram negative bacteria were highest resistant to oxacillin, amoxicillin, co-trimoxazole, cefuroxime, clarithromycin, azithromycin and Cefpodoxime whereas sensitive to colistin, tigecycline, levofloxacin, gentamycin, amikacin, imipenem and ampicillin and were high rate of resistance to Oxacillin and susceptible to Colistin, Tigecycline, Levofloxacin, Gentamycin

Conclusion: *Klebsiella pneumoniae* followed by *Acinetobacter baumannii* were the common infectious agents isolates in several gastrointestinal disorders.

Keywords: Gastrointestinal disease, *Klebsiella pneumoniae*, Gram Negative Bacteria, Gram Negative Cocci.

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Introduction

Gut microbiota is the complicated association of microorganisms which stay in the digestive tract or intestinal tract in both humans being and animals including insects. That of human gut contains about

10^{13} - 10^{14} bacteria that belong to hundreds of different species [1]. Gut microbiota plays a significant character in various metabolic, physiological, nutritional, and immunological processes in human host.

They cause different Gastrointestinal diseases like esophagitis, gastritis, gastric cancer, gastric ulceration, peptic ulcers, gastric mucosa, Helicobacter pylori infection, Peptic ulcers etc. Mostly diarrhoea occurs in the elderly age which is caused due to Clostridium difficile [2,3]. The virulent strains of Escherichia colicause gastroenteritis and Crohn's disease [4] whereas H. pylori have the capacity to cross the epithelial lining of the stomach and cause stomach ulcers. Antimicrobial susceptibility testing of bacteria can manage the adequate therapy and also provide an estimate antimicrobial efficacy[5]. The whole range of antibiotic resistant gene in some definite microbiota is identified in non-pathogenic and pathogenic bacteria. Management of antibiotics intent to cause dysbiosis come up with decrease in colonization which specifically produce resistome in microorganisms inside the intestine. Therapy over dominance of present increase of MDR (multi drug resistant bacteria) is very confined [6-11].

Advancement of drugs (antibiotics) being the preferred therapy for bacterial infections. Although the use of antibiotics triggers various troubles in medications of pathogenic infections since it can modify the working capacity of the gut microbiome that are dominant to other and such changes could have multiple physiological state outcomes. i.e. use of β -lactams & fluoroquinolones for 7 day, it was observed that the microbial diversity declining by 25% and core ancestral phylogenetic microbiota by 58.6% [12].

The pattern of infectious bacteria and there antimicrobial susceptibility test vary from country to country, as well as hospital to hospital. The lack of studies doesn't emphasize the susceptibility patterns of locally prevalent organisms. So this study assessed the prevalence of microorganisms causing gastrointestinal tract infections and their antimicrobial susceptibility pattern of

patients attending in our tertiary care hospital.

Material and methods

A prospective of six months study was carried out from March 2022 to December 2023, in the Department of Gastroenterology and Department of microbial Laboratory, IMS and SUM Hospital, under SOA deemed to be University, Bhubaneswar. Individuals attending to Department of Gastroenterology and admitted with various gastrointestinal disorders were included in the current study.

Sample collection and processing

Tissue samples collected from pylorus region of the patients during the Endoscopy procedures. The collected tissue samples were placed in 2ml test tubes containing Rapid Urease Test Broth (RUTB), which were freshly prepared. The collected samples were kept in the shaker inside the incubator at 37°C for 3 days within the 3 hours of collection. After 3 days the tissue samples were first cultured on Columbia Blood Agar Base (CBAB) plates and MacConkey (MAC) agar plate.

Then the cultured plates were incubated overnight at 37°C. Then after one day (24hrs) the cultured plates were observed and the results were noted. The microbial isolates were again pure cultured on NA (Nutrient Agar) plates to obtain single colonies for further tests and preservation. After observing colony morphology gram staining was done and identification was done by different biochemical tests.

Biochemical Tests

Biochemical experiments are helped in the identification of bacteria species. Usually basing upon the physiological activities of different bacteria, they show different biochemical activities in different chemical tests and that are vary species to species. So the different biochemical tests done were Catalase test, Oxidase test, Indole test, Methyl Red test, Voges Proskauer test,

Citrate test, Urease test and Triple Sugar Iron (TSI) test.

Antibiotic Susceptibility Test (ABST)

Antimicrobial susceptibility test was done by using Standard disc diffusion method i.e. Kirby-Bauer test. The test was done on freshly prepared Muller Hinton Agar (MHA) plates using sterile spreader. After the incubation of 24 hours the clear inhibition zones were measured using Hi Antibiotic Zone Scale.

Results

Total number of 95 samples was collected from the patient from whom 58 numbers of samples showed significant growth and 37 numbers of sampling showing no growth. Only bacterial growth found. More than half of the study participants whose cultural organism were identified were from males 40(69%) rest 18(31%) were female. Most of the isolates were from the age group of 21-40 years with a fraction of 31% followed by with a fractional distribution of 28% within the age group of 41-60 years were identified (Table 1).

Table 1: Number of individuals with their age ranges

| Sl. No | Age range | M | F | Total individuals | Percentage |
|--------|-----------|----|---|-------------------|------------|
| 1 | 0-20 | 3 | 1 | 4 | 7 |
| 2 | 21-40 | 12 | 6 | 18 | 31 |
| 3 | 41-60 | 11 | 5 | 16 | 28 |
| 4 | 61-80 | 9 | 4 | 13 | 22 |
| 5 | 81-100 | 5 | 2 | 7 | 12 |

Gram's staining result : In Gram's staining two different groups of bacteria were elucidated i.e., Gram Negative Bacteria (GNB) and Gram Negative Cocci (GNC) (Table 2).

Table 2: Gram Staining Result

| Serial No. | Category of organism | Frequency |
|------------|----------------------|-----------|
| 1. | GNB | 49 |
| 2. | GNC | 19 |

Gram staining figures of different individual samples were illustrated (Figure 1). Specifically the it was differentially observed GNB and GNC (Figure 1)

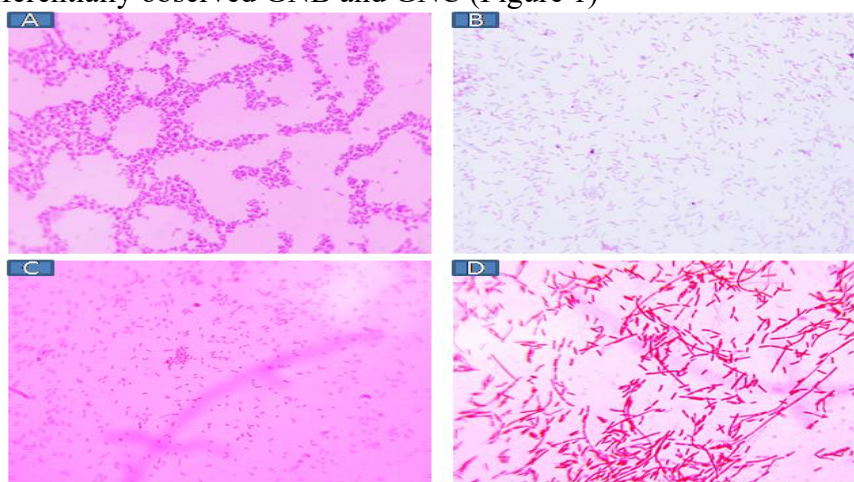


Figure1 (A-D): 1A an 1B and 1C representing GNC; 1B and 1D representing GNB

Result of total number of identified bacteria: A total of 58 numbers of 10 different types of bacterial species were

identified during the cultural process of bacterial isolation.

Isolates all were gram negative bacilli. Most prevalent GNB was *Klebsiella*

pneumoniae (21%) followed by *Acinetobacter baumannii* (17%) (Table 3). No gram positive coccus were found in the current cultural study; further out of the total cultured plates the *E. coli* were

observed in MAC plates, *K. oxytoca* observed o MAC plates, *C. freundii* was observed on NA plate and *K. pneumonia* was observed in NA plate.

Table 3: Total number of gram negative bacteria isolated their frequency and percentage

| Identified bacterial sp. | Number of isolates | Percentage |
|--------------------------------|--------------------|------------|
| <i>Klebsiella pneumoniae</i> | 12 | 21 |
| <i>Acinetobacter baumannii</i> | 10 | 17 |
| <i>Proteus vulgaris</i> | 9 | 15 |
| <i>Pseudomonas aeruginosa</i> | 8 | 14 |
| <i>Enterococcus aerogenes</i> | 7 | 12 |
| <i>Proteus mirabilis</i> | 4 | 7 |
| <i>Klebsiella oxytoca</i> | 4 | 7 |
| <i>Citrobacter freundii</i> | 2 | 3 |
| <i>Escherichia coli</i> | 1 | 2 |
| <i>Serratiamarcescens</i> | 1 | 2 |

Antibiotic susceptibility test for gram negative bacteria revealed that *Escherichia coli* had highest rate of resistance. Though *Klebsiella pneumoniae* was highest isolates, but the percentage of resistance is less than *Acinetobacter baumannii* and *Proteus vulgaris*. Drug susceptibility pattern revealed highly resistant to oxacillin and azithromycin whereas colistin was sensitive to all (Figure 2).

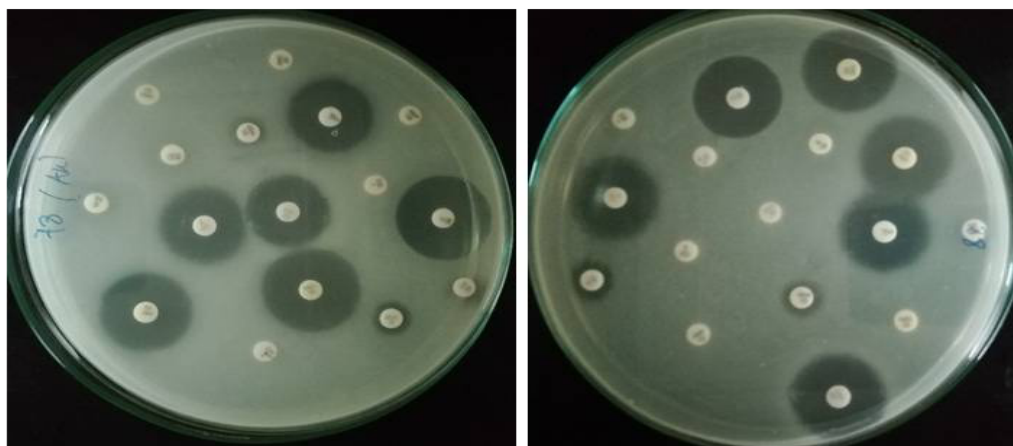


Figure 2: Antibiotic Susceptibility test of Gram Negative Bacteria

Discussion

Human gastrointestinal tract consist of a billion of bacteria, viruses and fungi, of which bacteria are the most infectious agnents which lead to severe gastritis, gastroenteritis, peptic ulcers, gastric cancer etc.

In a study it was found that among 2 billion gastroenteritis cases 1.3 million deaths occurred globally in 2015. In 2011 there

were 1.7 billion cases out of which 0.7 million were dead. Fatalities were more than 450,000 and children under 5 years old were affected due to rotavirus. It is less commonly affected the adults due to Acquired immunity and mostly seen in 2 to 5 years old children.

In this surveillance study, patients among age group of 21-40 were highest among all other age groups. In gastroenteritis number

of female patients was less than male patients. So it is cited that gastroenteritis is more possibly occur in male than female.

According to Gram staining result Gram negative bacteria (GNB) were more frequent than Gram negative cocci (GNC) than Gram positive bacteria (GPB). According to this study *Klebsiella pneumoniae* (21%) was most common isolated followed by *Acinetobacter baumannii* (17%), *Proteus vulgaris* (15%), *Pseudomonas aeruginosa* (14%), *Proteus vulgaris* (7%), *Enterococcus aerogenes* (12%).

In a study it was observed that a total number of 250 enterococci isolates, from which 174 (69.6%) were ampicillin resistant, 187 (74.8%) to gentamycin, 240 (96.0%) to ciprofloxacin, 57 (22.8%) to vancomycin and 56 (22.4%) to teicoplanin. Among urinary isolates, 42 (32.8%) and 118 (92.2%) isolates showed resistance to nitrofurantoin and norfloxacin respectively. Single isolate was resistant to linezolid, whereas all isolates were susceptible to tigecycline by disk diffusion method. It was found that *Enterococcus faecium* was more resistant than *Enterococcus faecalis* [13].

In this study we found that all gram negative bacteria were highest resistant to Oxacillin (92.28%), Amoxicillin (70.47%), Cefuroxime (73.97%), Cefpodoxime (77.64%) whereas sensitive to Colistin (96.95%), Tigecycline (97.29%), Levofloxacin (93%), Gentamycin (96.5%), Amikacin (94.5%), Imipenem (95.79%) and Ampicillin (94.41%). *Escherichia coli* had highest percentage of resistance than other bacteria. Whereas *Klebsiella pneumoniae* being highest isolates showed a least resistant than *Acinetobacter baumannii* and *Proteus vulgaris*.

Conclusion

The majority of the gastroenteritis was more in male patients than female patients. The percentage of male patient was 69% and female patients were 31%. From total number of 100 samples only 68 samples showed significant growth. The

predominant isolated bacteria were *Klebsiella pneumoniae* (21%) followed by *Acinetobacter baumannii* (17%), *Proteus vulgaris* (15%), *Pseudomonas aeruginosa* (14%), and *Enterococcus aerogenes* (12%), *Proteus mirabilis* (7%), and *Klebsiella oxytoca* (7%), *Citrobacter freundii* (3%), and *Escherichia coli* (2%), *Serratia marcescens* (2%).

Klebsiella pneumonia was highest isolates, but the percentage of resistance is less than *Acinetobacter baumannii* and *Proteus vulgaris*. *Escherichia coli* had highest rate of resistance. High rate of resistance to Oxacillin (92.28%) by all gram negative bacteria. The susceptibility rates to different antibiotics were Colistin (96.95%), Tigecycline (97.29%), Levofloxacin (93%), Gentamycin (96.5%), Amikacin (94.5%), Imipenem (95.79%) and Ampicillin (94.41%). Nowadays, resistance too many of the commonly used antibiotics in ICU set-ups, is posing a problem in the clinical scenario.

Moreover cross-infection inside a hospital gives rise to notoriously resistant strains. *Klebsiella pneumoniae* and *Acinetobacter baumannii* are typical bacteria which are stands out among all bacteria in present critical care units.

Formulating local antibiotic policies, guided by the nature of bacterial isolation and their antibiotic susceptibility patterns may help the clinicians to offer more effective empiric treatment.

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