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ORIGINAL ARTICLE

Superintendence of antimicrobial resistance observed in bacterial flora isolated from human faecal carriage in Vellore, India

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Abstract A frequent cross-sectional study was conducted to determine the patterns of antimicrobial resistance in 296 bacterial strains isolated from in-patient faecal samples of Government Vellore Medical College and Hospital, Vellore. Isolation and identification of bacterial strains were done using enrichment media, selective media, and biochemical tests. Antimicrobial susceptibility testing by the disc diffusion method and minimal inhibitory concentration method was conducted and the strains were subjected to extended spectrum beta-lactamases screening. Antibiotic sensitivity pattern of *Staphylococcus* spp. showed oxacillin resistance. Almost all the strains were sensitive to linezolid, vancomycin, gentamycin and chloramphenicol. In gram negative isolates ciprofloxacin and tobramycin showed better sensitivity and ceftazidime showed a higher percentage of resistance by MIC. Out of 250 isolates, Enterobacteriaceae showed positive for 86/250, 82/250 and 94/250 isolates and 3/10, 4/10 and 4/10 non-Enterobacteriaceae isolates were found to be positive for CTX-M gene, TEM gene and SHV gene, respectively. This study helps to assess/analyse the relation between the spectrum of microorganisms present in various grades of faecal carriage and their susceptibility pattern in this part of the Vellore town.

1. Introduction

Antibacterial resistance has been recognized as an up-and-coming worldwide problem in both human and animals, and antibacterial agent use is considered the most important factor for the emergence, selection, and dissemination of antimicrobial agent-resistant bacteria (Prescott et al., 2000). The principle behind the development of resistance is that

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bacteria in the guts of humans are subjected to different types, concentrations, and frequencies of antimicrobial agents. Over time, selective pressure selects resistant bacteria that have specific fingerprints for resistance to the antimicrobial agents that have been used. On other hand, contamination of the hospital environment leads to the development of the antimicrobial agent resistance.

Worldwide, there are increasing problems with multiresistant bacteria. The most important drug resistance today is caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (Udo et al., 2001; Borg, 2003), vancomycin resistant enterococci (VRE) (Calfee et al., 2003; Kuriyama et al., 2003) and Enterobacteriaceae with extended-spectrum beta-lactamases (ESBL). The common aerobic pathogens isolated from the chronic wounds are *S. aureus*, coagulase negative *Staphylococcus* (CONS), *S. epidermidis* and *Streptococcus* spp. Recent clinical attention has focused on the increasing frequency of non-lactose fermenting gram-negative pathogens responsible for hospital-acquired infections, *Pseudomonas aeruginosa* and coliform bacteria (Armstrong et al., 1995). Multidrug resistant organisms (MDROs) in the foot and hand ulcer infection may increase the duration of hospital stay, an increase in cost of management and might lead to additional morbidity and mortality (Gadepalli et al., 2006). The first ESBL isolates were discovered in Western Europe in the mid 1960's. They were found in a variety of Enterobacteriaceae spp. Most beta lactamases function by a serine ester hydrolysis mechanism, rendering the antibiotics inactive. Beta-lactamases of gram negative species are periplasmic and protect the cell (Thomson and Sanders, 1992).

Resistance to broad-spectrum cephalosporins has emerged in strains of members of the family *Enterobacteriaceae* following frequent use of these drugs in the hospital setting. Endemic and epidemic nosocomial infections caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* cells represent a persistent problem in many parts of the world (Komatsu et al., 2003). Epidemic strains of cephalosporin-resistant *E. coli* and *K. pneumoniae* have been associated with increased morbidity and mortality in hospitalized patients. Appropriate isolation measures need to be taken, hand hygiene procedures may need reinforcement, possible environmental reservoirs need elimination, and antibiotics policies may need reconsideration (Gruteke et al., 2003; Muzaheed Doi et al., 2008).

To test this hypothesis, the two objectives of this study were (i) to identify patterns of antimicrobial agent resistance of bacterial strains obtained from the faecal carriage of in-patients of Government Vellore Medical College and Hospital, Vellore (ii) to subject Enterobacteriaceae and non-Enterobacteriaceae bacteria to ESBL screening.

2. Materials and methods

2.1. Sample collection

Specimens (faecal samples) for microbiological studies were obtained from in-patients admitted in different wards of the Government Vellore Medical College and Hospital (GVMCH), Vellore. Faecal samples were collected in plastic universal containers that were immediately put on ice. Clinical details of the patients were also collected. The transport media (Muller Hinton broth) was used for specimen transport to our laboratory.

2.2. Sample processing

A total of 187 faecal samples were collected. Direct examinations of the specimen provided immediate semi-quantitative information about the types of organisms present, and other distinguishing characteristics of the specimens were also noted.

A direct smear was made from the specimen, and inoculated into appropriate aerobic plating media, such as Blood agar and MacConkey agar. The inoculated plates were immediately incubated under aerobic condition at 37 °C for 24 h, respectively. The direct smear was dried, fixed, and gram stained. The gram-stained smear was examined for information about the types of organism present. The aerobic plates were examined after 24 h of incubation. The isolated microorganisms were identified using standard methods. Identification of isolates were done based on colony morphology, gram staining, motility, catalase test, oxidase test, coagulase test, standard biochemical tests and oxidation-fermentation test and other tests (Collee et al., 1996).

Each isolate was grown overnight (18–20 h) on Blood agar or MacConkey agar. A single colony was made in the stock medium i.e., 5% trypticase soy broth plus 20% v/v. All the isolates were kept frozen at −70 °C until further use.

2.3. Antimicrobial susceptibility test by disc diffusion method

All the isolates tested for antimicrobial susceptibility against antimicrobial agents. Gram-positive bacteria were tested against the various antibiotics such as ampicillin (A) – 10 μg, chloramphenicol (C) – 30 μg, ciprofloxacin (Cf) – 5 μg, linezolid (Lz) – 10 μg, penicillin G (P) – 10 units, streptomycin (S) – 10 μg, vancomycin (Va) – 30 μg, oxacillin (Oxa) – 30 μg, gentamycin (G) – 10 μg and co-trimoxazole (Co) – 25 μg disc. Gram-negative bacteria isolated from samples were tested against various antibiotics such as amikacin (Ak) – 30 μg, ampicillin (A) – 10 μg, aztreonam (Ao) – 30 μg, cefepime (Cpm) – 30 μg, ceftazidime (Ca) – 30 μg, cephotaxime (Ce) – 30 μg, chloramphenicol (C) – 30 μg, ciprofloxacin (Cf) – 5 μg, gentamycin (G) – 120 μg, imipenem (I) – 10 μg, nalidixic acid (Na) – 30 μg, norfloxacin (Nx) – 10 μg, tetracycline (T) – 30 μg, trimethoprim (Tr) – 5 μg, ticarcillin (Tc) – 7.5 μg and piperacillin/tazobactam (Pt) – 30 μg disc. *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *K. pneumoniae* ATCC 70063, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC11775 were used as control strains.

2.4. Antimicrobial susceptibility test by micro-broth dilution method

The stock solution of oxacillin, cephotaxime, ceftazidime and imipenem drugs were prepared dilution range of 1–64, 1–256, 1–256 and 1–256 respectively, according to the CLSI (2006) recommendation. Inoculum of the test organisms were prepared from colonies grown on Nutrient agar (Hi-media), which had been incubated overnight (18–20 h) at 37 °C in the incubator. Colonies were suspended in Mueller Hinton broth (Himedia) and adjusted to a turbidity of a 0.5 McFarland standard (1×10^8 CFU/ml).