Antibiogramme Pattern Of Diarrhoegenic E.Coli

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Summary. One hundred and forty nine isolates, obtained from infant diarrhoeal patients provided by General Hospital Kuala Lumpur (GHKL) were examined to determine their biochemical and bacteriological characteristics. All 149 isolates were identified as Escherichia coli and screened for 0157:H7 strains based on β-glucuronidase activity using MUG plate which was a rapid method for VTEC identification (1,2). Antibiotic sensitivity study was done in order to determine which antibiotics showed high resistance among isolated strains and whether E.coli strains were resistance towards a single antibiotic or multiresistance. From the study, it was found that 87.92% strains which was the highest percentage, showed resistant towards cephalothin and there were as many as 17 different antibiogroup patterns observed among the strains.

Introduction

E.coli is presence in the intestine and plays an important role in maintaining the physiology of the intestine. It is a pathogen of man and animals, and causes both septic infection and diarrhea (3). Bray (4) succeeded in showing by serological methods that E.coli was the etiological agent responsible for causing diarrhoea. Between 1950 its role as an enteric pathogen was highlighted (5) and thus, E.coli is no longer viewed as a mere opportunistic, but special strains that can be considered as primary pathogen possessing an array of virulence traits that allow the organism to evade host defences and cause overt disease. The isolation of E.coli from faeces is now a fairly simple procedure which can be done by most hospital laboratories. E.coli can be isolated from faecal specimens by using selective media (eg Eosin Methiyene Blue agar, Mac Conkey agar, Bismuth Sulphite agar etc.) that reduce the growth of competing microorganisms. E.coli is a negative straight rod bacteria, with size ranging from 1.1-1.5μm by 2.0-6.0μm for living organism and 0.4-0.7μm by 1.0-3.0μm for dried and stained organism (6). Many strains have capsules or similar less well developed structures (microcapsules). E.coli is normally susceptible to a variety of the broad spectrum antibiotics, including tetracycline, chloramphenicol, ampicillin, erythromycin, neomycin and streptomycin as well as sulfonamides. However, drug resistance is increasingly prevalent among isolated strains, and a major portion of these carry R plasmids directing resistance to one or more of the drugs. Although results from different surveys are somewhat variable, resistance to ampicillin, streptomycin, tetracycline, chloramphenicol and sulfonamides appear to be the most common. In 1968, Gunter & Feary has reported that multiple resistance also occurs with high frequency among strains isolated from hospital patients and 70.5% were resistant to more than 1 drug (7). The aim of this study was to evaluate the biochemical characteristics of having β-glucuronidase activity which was applied for E.coli determination and differentiation of VTEC from other types. Also, this study was mean to determine the antibiotic susceptibility test towards selected antimicrobial agents in order to obtain a pattern of resistance from E.coli isolates of diarrhoeal patients.

Materials & Methods

Bacterial strains. The E.coli strains used in this study were obtained from the General Hospital of Kuala Lumpur (GHKL). Plasmid-free E.coli ATCC 25922 was used as a sensitive control in all antibiotic sensitivity tests in this study.
**Preparation of cultures and bacterial suspension.** All *E. coli* strains which have been kept in 25% sterile glycerol as stocks at -70°C were reconstituted by plating onto Brain Heart Infusion (BHI) agar medium and incubated at 37°C for 18-24 hours. The colonies were then harvested and dispersed into BHI broth. The turbidity of the suspension was adjusted by adding 0.85% sterile saline until visually matched the Mc Farland 0.5 turbidity standard for use in antibiotic sensitivity test and MIC assays. Bacterial cultures were also maintained on nutrient agar slants for testing the β-glucuronidase activity.

**Analysis of β-glucuronidase activity** The β-glucuronidase activity was measured in 4-methylumbelliferyl-β-glucuronidase (MUG) agar plate (8). *E. coli* strains and ATCC 25922 were inoculated onto MUG agar and incubated 18-24 hours at 37°C before results could be obtained.

**Antibiotic Sensitivity Test.** The test was conducted by the disc diffusion method using the Kirby-Bauer test. The antibiotics used in this study were ampicillin (Am) (10μg, BBL), chloramphenicol (C) (30μg, BBL), cephalothin (CF) (30μg, BBL), erythromycin (E) (15μg, BBL), kanamycin (K) (30μg, OXOID), nalidixic acid (N) (30μg, OXOID), sulphamethoxazole/trimethoprim (Sxt) (25μg, OXOID) and tetracycline (Te) (30μg, OXOID). The test culture prepared as mentioned above was swab evenly on the ISO-sensitest agar using a sterile cotton swab, and allowed to dry for 5-10 minutes. Using a fine pointed forceps, antibiotic discs were placed onto the agar firmly. The plates were incubated invertedly at 37°C for 18-24 hours. Sensitivity response of the bacteria was observed as inhibited zone surrounding the discs. The degree of sensitivity was measured by the diameter of the inhibited zone and translated to prefixed susceptible (S), intermediate (I) or resistant (R) categories by referring to the interpretative chart (NCCLS, 1985) provided by the manufacturers. *E. coli* ATCC 25922 was used as sensitive control throughout this test. The test was repeated three times to ensure results accuracy.

**Minimal inhibitory concentration (MIC).** Cultures were prepared as above to a density of a Mc Farland 0.5 turbidity standard. Aliquots of 0.5 ml each well suspension was placed aseptically in the corresponding wells in the replicator seed block (9). With a multipoint inoculator, 2 μl of the bacterial suspension was applied to the surface of the Mueller Hinton antibiotic agar plates, each containing a different concentration of the chosen antibiotic. As control, *E. coli* ATCC 25922 was inoculated as control on each plate. All plates were incubated at 37°C for 18-24 hours following which the MICs were recorded as the lowest antibiotic concentration with no visible growth.

**Results And Discussion**

Fluorogenic procedure with MUG substrate is a common method for *E. coli* identification from human specimen (10,11,12,13,14) because 96% of *E. coli* produce β-glucuronidase enzyme (10,14) which cleaves the MUG substrate methylumbellifereone and this product will light up under uv light (15,16). In this study, all strains gives MUG-positive results thus confirmed that all *E. coli* strains used in this study were not 0157:H7 because strains of serogroup 0157:H7 will give a consistent MUG negative reaction result (1,2,10,14).

The Kirby-Bauer test screened the *E. coli* strains for their antibiotic susceptibility towards 8 different antibiotics. The frequency of antibiotic resistant among the *E. coli* strains is shown in Figure 1. Although *E. coli* is normally susceptible to a wide variety of wide spectrum antibiotics, drug resistance is increasingly prevalent among isolated strains. In particular, most strains in this study are sensitive to nalidixic acid, chloramphenicol and kanamycin but are resistant to common antibiotics tested which shows agreement with the study reported by Freeman, 1979 (17). Seventeen different antibiotic groups were displayed by the strains (Figure 2). Multiple drug resistances were evident in the isolates (97%), except 3% showed resistance to a single antibiotic of cephalothin and 2% were susceptible to all antibiotics tested, thus did not fall into any of the antiobigroup.
The minimal inhibition concentration determined the minimal concentration of antibiotics to inhibit bacteria growth. The strains showed a variety of MIC values towards antibiotic tested (Figure 3). Therefore it appears that diarrhoeagenic E.coli strains in Malaysia are generally resistant to antibiotics tested.
Figure 3: MIC values of antibiotics of the E.coli isolates

References