Study on Antibiotic Resistance by Pathogenic Bacteria Isolated from Clinical Specimen

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Abstract: An attempt was made to isolate and study the resistance of bacterial pathogens from clinical specimens (stool, blood and skin lesion) of hospitalized patients against three antibiotics viz., erythromycin, tetracycline and penicillin G. Seven different strains of pathogenic bacteria viz., Salmonella typhi, S. paratyphi, A. shigella dysenteriae, S. sonnei, Staphylococcus aureus, Enterobacter aerogenes and Escherichia coli were isolated. All the isolates were found resistance to penicillin G and tetracycline except S. typhi and S. sonnei which were found sensitive to tetracycline. On the other hand, all the isolates were found to be sensitive against erythromycin tested herein and the highest zone of inhibition (26 mm) was recorded against S. typhi. Subsequent agarose gel electrophoresis showed no plasmid-DNA band in the gel indicating that observed resistance was chromosomal gene-mediated.

Key words: Antibiotic resistance, pathogenic bacteria, clinical specimens

INTRODUCTION

Antibiotics are metabolic products of microbial-origin widely used as chemotherapeutic agents for their power of being, even in very small amounts detrimental or inhibitory to pathogenic microorganisms. Antibiotic resistance is that situation when a particular antibiotic drug can’t kill or inhibit a pathogenic microorganism which was sensitive to that particular drug before. Now, it is a global problem. The first bacterium that showed resistance to penicillin was Staphylococcus aureus in 1943. Now only approximately 5% of Staph. aureus isolates still are susceptible to penicillin. Strains that are oxacillin and methicillin resistant, historically termed methicillin resistant S. aureus (MRSA) are resistant to all β-lactam agents including cephalosporins and carbapenems plus erythromycin, clindamycin and tetracycline.

In India multi-drug resistant, Shiga-Toxin-producing Escherichia coli (STEC) non-0157 has been reported. Vibrio strains isolated in Bombay have been reported to be resistant to ampicillin, streptomycin and tetracycline. Antibiotic-resistant Salmonella enterica serovar Typhimurium and Salmonella enterica serovar Albany have been reported previously. About 12.9% Salmonella enteritidis human strains are resistance to antibiotic have been reported. Resistant veterinary isolates of Salmonella enteritidis has been reported in Malaysia. 62.6% E. coli isolates from urinary tract infections were found resistance to amoxicillin (1999-2000) in Tunisia. About 50% cephalosporin-resistance by Aeromonas hydrophila strains in infected fish in Bangladesh was reported. A highly ceftriaxone-resistant Salmonella typhi in Bangladesh was also reported. Resistance by Klebsiella pneumoniae, K. oxytoca and E. coli to cephalosporins have also been reported. Resistance may spread from one species to another. The product of mar operon, which was initially found in E. coli, cause the resistance to at least eight antibiotics and disinfectants (triclosan, quaternary ammonium compounds) possibly by decreasing uptake combined with increased efflux. The mar operon was also found in six other enterobacteriaceae: Salmonella, Shigella, Klebsiella, Citrobacter, Hafnia and Enterobacter species. Bangladesh is a third world country where use of antibiotic is not controlled both in dose and duration. So, the present research has been taken to isolate and study the resistance of bacterial pathogens of hospital patients against three antibiotics.

MATERIALS AND METHODS

Well-preserved specimens e.g. stool, blood, skin-lesion from hospitalized patient were examined in a diagnostic laboratory (privately-owned) near Chittagong Medical College. Pre-enrichment, primary isolations and serological tests were done. Biochemical tests were done in the Department of Microbiology, University of...
Chittagong, Bangladesh. The specimens were kept in sterile containers at 4°C before isolation and identification.

**Isolation of Salmonella:** One milliliter blood sample was mixed with 10 mL of sterile buffered peptone water and incubated at 37°C for 24 h. Then 1 mL of pre-enriched culture was added to 10 mL of Muller-Kauffmann tetraphionate broth (MK-TB), mixed well and then incubated at 43°C for 48 h. Subculture was made from surface of MK-TB to Brilliant Green Agar (BGA) and Xylose Lysine Desoxycholate agar (XLD) and incubated at 37°C for 22-24 h. In BGA plates Salmonella colonies were pink, smooth and low convex. In XLD plates Salmonella colonies were red with black centres and 3-5 mm diameter.

Suspected colonies were subjected to subsequent gram staining (gram negative short rod), biochemical and serological tests to identify the strain.

**Isolation of Shigella:** A loopful of stool was mixed with 10 mL of sterile buffered peptone water and incubated at 37°C for 24 h. After incubation a loopful of culture was streaked on the MacConkey agar plates and were incubated at 37°C for 24 h. Non-lactose fermenting colonies (i.e. colorless) on MacConkey agar plates were inoculated on XLD agar and incubated at 37°C for 24 h. After incubation, red colonies with 2-4 mm diameter were marked and suspected colonies were subjected to subsequent gram staining (gram negative short rod), biochemical and serological tests to identify the strain.

**Isolation of E. coli:** A loopful of stool specimen was streaked on MacConkey agar plates and incubated at 37°C for 24 h. Pinkish colonies with 2-3 mm in diameter and Gram negative cells were observed. Identification was done by biochemical tests.

**Isolation of Enterobacter aerogenes:** One loopful of stool specimen was plated on MacConkey agar plates and incubated at 37°C for 24 h. Lactose-fermenting (i.e. pink to red), large and mucoid colonies and gram negative rod cells were selected for biochemical tests. Identification was done by biochemical and physiological tests.

**Isolation of Staphylococcus aureus:** Pus from skin lesion was plated on blood agar plates, incubated at 37°C for 24 h. Circular, golden-yellow, opaque, domed colonies with around 2 mm diameter were observed. No beta-hemolysis developed. Gram positive, coccoid cells in grapelike clusters were seen under microscopic observation. Identification was done by biochemical and physiological tests.

Antibiotic sensitivity tests were done 3 times for each strain by disc diffusion method\[^{13}\]. Then, Alkaline-lysis method used for plasmid-extraction (if they were present at all) and agarose gel electrophoresis was done by the method described by Sambrook\[^{19}\]. All the cultures physiological and biochemical tests for identification of pathogenic bacteria were done.

**RESULTS AND DISCUSSION**

To identify the isolates obtained from different hospitalized patients were tested for various biochemical, physiological and serological characters. According to the results of Table 1 the isolates are closely related to Salmonella typhi, S. paratyphi-A., Shigella dysenteriae, S. sonnet, Staphylococcus aureus, Enterobacter aerogenes and Escherichia coli.

Enterobacter aerogenes and Salmonella typhi showed two types of response to penicillin G: complete inhibition and partial inhibition (Table 2). Those cells which were completely inhibited, failed to resist the action of corresponding antibiotics against them and so they became sensitive to the antibiotics. But partial inhibition happened when somehow some mutant cells emerged and they could successfully resist the action of the antibiotics against them. Such phenomenon has been reported by some workers\[^{14}\]. Staphylococcus aureus strain was found resistant against penicillin G and tetracycline. The *Salmonella typhi* strain was found resistant against β-lactam antibiotic, penicillin. Results of the studies in near past comply with the observation\[^{25-26}\]. Multi-drug resistance by *E. coli* was also not uncommon\[^{10}\]. Resistance by *Shigella* sp. was also not uncommon\[^{2}\].

Plasmids were not found in the resistant strains. This was not unnatural, because a multi-drug resistance regulatory chromosomal locus, mar, has been reported\[^{10}\] to be widespread among enteric bacteria. Presence of an emr gene (on *E. coli* chromosome) that codes for protein products of membrane’s translocate family and a *E. coli* locus for multi-drug resistance have also been reported\[^{9}\]. Besides, differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of mar-A has been reported by workers. They found that such expression gives rise to multiple antibiotic resistance by *E. coli*\[^{10}\]. So the antibiotic resistance observed in this study can be thought to be chromosomal gene-mediated, i.e. not plasmid-mediated.

To counter the potential problem we should avoid using antibiotics unnecessarily (i.e. in case of curing common flu or viral diseases). We should use the most specific antibiotic possible. "Narrow-spectrum,"
antibiotics will only, agitate and, kill the offending strain. We should use the common antibiotics first. Then we should reduce hospital-transmitted infections, with ultraviolet lights, better sanitation and putting patients with recalcitrant infections in isolation wards. Ideally, two new drugs could be used at once, to slow the development of resistance.

REFERENCES


