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Study of plasmid linked resistance pattern of MDR enterobacterial pathogens

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Abstract

Ten multiple drug resistant enterobacterial pathogens were isolated from common sources of human consumption and were subjected to plasmid isolation. The multiple drug resistant (MDR)(write full abbreviation) isolates collected in the present study indicated the presence of plasmids of almost identical size of approximate 60 kb conferring resistance towards multiple antibiotics. An opportunistic pathogen *Pseudomonas aeruginosa* contained a plasmid of slightly higher molecular weight. The isolates were subjected for antibiotic susceptibility testing against 10 antibiotics and found to be resistant towards six antibiotics. All the isolated MDR pathogens were found to be sensitive towards amikacin. Sensitivity for cefuroxime, oxacillin, and metronidazol differed for different species. The pattern of MDR bacteria was perturbing as simultaneous resistance to chloramphenicol and gentamycin, formed the common MDR pattern. The pattern was almost the same for the diverse species (*Escherichia coli, Klebsiella pneumoniae, Citrobacter diversus, Shigella flexneri, Salmonella typhi, Proteus vulgaris, Citrobacter freundii, Proteus myxofaciens, Klebsiella oxytoca*, and *Pseudomonas aeruginosa*) isolated from different food samples and strongly suggests prevalence of similar R plasmids. This suggests that antibiotic resistance is encoded on a high molecular weight plasmid, and can easily spread in the community through food stuff generally consumed by the common man.

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Introduction

The observations of resistance development in *Shigella* to sulpha drugs in 1940s and to tetracycline and chloramphenicol in 1950s followed by subsequent *Shigella* dysentery outbreak of 1954 in Japan, has attracted word-wide attention of scientific community. Since then, very large number of publications appeared in the literature concerning the development of drug resistance in a variety of microbes present in food items, fruit juices, sweets, sprouts, milk and milk products, fresh and marine waters. After the involvement of extra–chromosomal autonomous genetic elements, subsequently referred as R-plasmids, responsible for multiple drug resistance (MDR) as demonstrated by Watanbe (1963), the researches in this area assumed new dimensions of molecular biology. The fear of transfer of multidrug resistance to pathogens like *Salmonella typhi* came true in 1972 resulting in an epidemic of chloramphenicol resistant *S. typhi* and in 1992 another epidemic with simultaneous resistance for chloramphenicol, co-trimoxazole and ampicillin (Chitnis *et al.,* 2000). *Salmonella sp*. is responsible for an estimated 0.8 to 4.0 million infections in the United States each year usually taking the form of gastroenteritis following the consumption of contaminated food. The incidence of resistant bacteria in foodstuff is a worldwide phenomenon. It is a major public health threat (Rahman and Malik, 2001).

The occurrence of transferable drug resistance mediated by plasmids, called R factor, has been extensively documented since it was first observed in Japan in 1959. Mandal *et al.* (2004) investigated the occurrence of R-plasmids among MDR isolates of *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae* from different clinical cases isolated from in and around Calcutta, India. The multidrug resistant *S. typhi* strains contained a transferable plasmid conferring resistance to ampicillin, chloramphenicol, cotrimoxazole and tetracycline. The plasmid encoding, ACCoT-resistance of *E. coli, Klebsiella pneumoniae* and *Proteus vulgaris* were conjugative and co-migrated with the plasmid of MDR *S. typhi* isolates.

A very rapid adaptation of bacterial populations under different selective pressures is a commonly observed phenomenon. The basis of antibiotic resistance development is due to mobile genetic elements such as plasmids and transposons. Plasmid mediated resistance to various antimicrobial drugs have been repeatedly demonstrated by plasmid curing experiments. The selection of resistant mutant strains and the transfer of mobile genetic determinants like plasmids and transposons, promoted increased antibiotic resistance (Spengler *et al.,* 2003). Some contributing factors include cross transmission, inter hospital resistance transfer, a community contribution to resistance and the use of a variety of antibiotics. The spread of antibiotic resistance among pathogenic bacteria thus posed a serious problem of therapeutic failure during the treatment of infectious diseases. The adaptation to antibiotics present in the aqueous environment is due to acquisition and dissemination of simple antibiotic resistance genes by mobile genetic elements (Cruz and Davies, 2000). As described by Gaynes and Monnet (1997), some contributing factors include cross transmission, inter hospital resistance transfer, a community contribution to resistance and the use of a variety of antibiotics.

The present study aims at isolation and characterization of MDR enterobacterial pathogens from various food samples. These samples are consumed by the common man in India, thru which infection could occur which could be difficult to cure due to their multiple drug resistant nature.

Materials and methods

Ten MDR pathogens were isolated from various sources including food items consumed by the common man. All organisms were isolated on chlorempheniсol and gentamycin supplemented MacConkey agar and identified as members of

Enterobacteriacae. Antibiotic susceptibility testing was conducted on Muller-Hinton agar using disc diffusion method described by Bauer *et al.* (1966) using commercial antibiotic discs purchased from Hi-media Laboratories. The discs were placed on Muller-Hinton agar previously seeded with 0.5 ml test culture grown for 24 h. The antibiotics used were Nalidixic acid, Gentamycin, Chloramphenicol, Metronidazole, Ciprofloxacin, Amikacin, Norfloxacin, Oxacillin, Cefuroxime and Ampicillin. The antibiotic resistance pattern was recorded after incubation of plates at 37 °C for 48 h.

Isolation of plasmids

Plasmids were isolated form the strains using standard alkaline lysis method with some modifications. After adding TSE buffer, enzyme treatment was given using 2.5 ml lysozyme as described by Sambrook *et al.* (1989), incubated at 37 °C for 30 min. The solution was then incubated at 65 °C for 30 min (Kado and Liu, 1981) in a water bath. Then the routine protocol was followed. The plasmid DNA in the supernatant was precipitated and incubated at –20 °C overnight. The plasmids thus obtained were subjected to gel eletrophoresis.

Results

Table 1 demonstrates the antibiotic resistant phenotypes of MDR pathogens used in the study. The organisms were found to be resistant towards almost all the antibiotics. All the isolated MDR pathogens were found to be sensitive towards amikacin. Sensitivity for cefuroxime, oxacillin, and metronidazol differed for different species.

Fig. 1 and 2 shows the electrophorogram of plasmids extracted from ten MDR pathogens isolated during the present study and a similar preparation from chloramphenicol and gentamycin sensitive strain of *Escherichia coli* DH 5α. The MDR isolates of *Escherichia coli, Klebsiella pneumoniae, Citrobacter diversus, Shigella flexneri* and *Citrobacter freundii* indicated the presence of plasmids of almost identical size of about 60 kb, whereas *Escherichia coli* DH 5α was devoid of any plasmid [Fig. 1]. The MDR isolates *Proteus vulgaris, Proteus myxofaciens, Salmonella typhi, Klebsiella oxytoca* showed the presence of similar plasmid of 60 kb (Fig. 2). *Pseudomonas aeruginosa* indicated the presence of slightly higher molecular weight plasmid (Fig. 2).

Table 1. Antibiotic resistant phenotypes of enterobacterial pathogens

Genus and species	Sensitivity pattern	Resistance phenotypes
Escherichia coli	Ami	Cip, N A, Amp, **Cef, Oxa, Met**, Gen, Chl, Nor
Klebsiella pneumoniae	Ami, **Cef, Oxa**	Cip, N A, Amp, **Met**, Gen, Chl, Nor
Pseudomonas aeruginosa	Ami	Cip, N A, Amp, **Cef, Oxa, Met**, Gen, Chl, Nor
Citrobacter diversus	Ami	Cip, N A, Amp, **Cef, Oxa, Met**, Gen, Chl, Nor
Shigella flexneri	Ami	Cip, N A, Amp, **Cef, Oxa, Met**, Gen, Chl, Nor
Klebsiella oxytoca	Ami , **Cef**	Cip, N A, Amp, **Oxa ,Met**, Gen, Chl, Nor
Salmonella typhi	Ami, **Cef**	Cip, N A, Amp, **Oxa, Met**, Gen, Chl, Nor
Proteus vulgaris	Ami	Cip, N A, Amp, **Cef, Oxa, Met**, Gen, Chl, Nor
Citrobacter freundii	Ami, **Cef**	Cip, N A, Amp, **Oxa, Met**, Gen, Chl, Nor
Proteus myxofaciens	Ami, **Oxa, Met**	Cip, **Cef**, N A, Amp, Gen, Chl, Nor

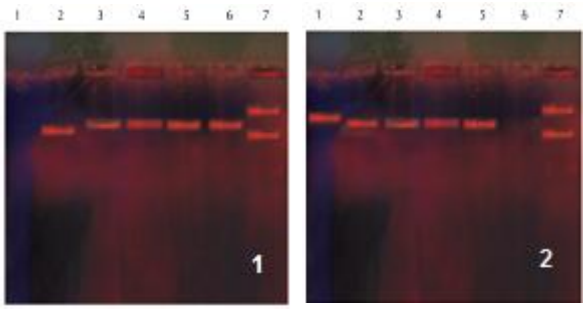

Fig. 1 and 2. Plasmid DNA isolated from MDR pathogens. Fig. 1: Lane 1-Escherichia coli DH 5α(drug sensitive), Lane 2 - Escherichia coli, Lane 3 - Klebsiella pneumonia, Lane 4 - Citrobacter diversus, Lane 5 - Shigella flexneri, Lane 6 - Citrobacter freundii, Lane 7 - Control (50 kb, 70 kb). Fig. 2: Lane 1 - Pseudomonas aeruginosa, Lane 2 - Proteus vulgaris, Lane 3 - Proteus myxofaciens, Lane 4 - Salmonella typhi, Lane 5 - Klebsiella oxytoca, Lane 6 - Blank, Lane 7 - Control (50 kb, 70 kb).

Discussion

Maximum number of isolates was found to be associated with the swabbing of tea glasses and plates, and found to contain *Escherichia coli, Klebsiella pneumoniae, Citrobacter diversus* and *Proteus vulgaris*. The next major source of MDR pathogens was water sprinkled on vegetables by vegetable vendors and found to contain *Escherichia coli, Citrobacter diversus, Shigella flexneri, Citrobacter freundii, Klebsiella oxytoca* and *Pseudomonas aeruginosa*.

In the aqueous environment, the transfer of resistance factor within and between the bacterial genera has been shown by Grabow and Prozesky (1973) using *Escherichia coli* resistant to ampicillin, chloramphenicol, sulfonamide and tetracycline. The MDR isolates collected during the present study indicated the presence of plasmids of almost identical size of approximate 60 kb, however an opportunistic pathogen *Pseudomonas aeruginosa* contained a plasmid of slightly higher molecular weight. *Escherichia coli* has been repeatedly shown to contain plasmid for resistance to a variety of antibiotics (Krueger *et al.,* 2008, Vigil *et al.,* 2009). Presence of plasmids in *Klebsiella pneumoniae* has been reported by several workers (Elizabeth and Vincent, 2010; Cunha *et al.* , 2008). The occurrence of R-plasmid in *Salmonella typhi* isolates was investigated by Mandal *et al.* (2004). Presence of plasmid in *Citrobacter spp.* was reported by Pepperell *et al.* (2002).

The pattern of MDR bacteria was perturbing as simultaneous resistance to chloramphenicol and gentamycin, formed the common MDR pattern. The pattern was almost the same for the diverse species (*Escherichia coli, Klebsiella pneumoniae, Citrobacter diversus, Shigella flexneri, Salmonella typhi, Proteus vulgaris, Citrobacter freundii, Proteus myxofaciens, Klebsiella oxytoca*, and *Pseudomonos aeruginosa*) and strongly suggests prevalence of similar R plasmids. These results can be co-related with the antibiotic patterns of the isolates. Almost similar antibiotic resistance pattern was observed for all isolates. These results suggest that antibiotic resistance is encoded on a high molecular weight plasmid as also suggested by Chitnis *et al.,* (2000). They reported similar MDR pattern in diverse species and presence of similar plasmid DNA bands on gel electrophoresis and demonstrated the presence of high molecular weight R plasmids (>10 kb) in MDR isolates obtained from hospital effluents. Antibiotic resistance associated with high molecular weight plasmids has been documented by several workers (Rajaa and Abdelaziz, 2001; Joshi *et al.,* 2003; Wain *et al.,* 2003 and Rajini *et al.,* 1992).Write about the future prospect of your findings? These results suggest that antibiotic resistance is encoded on a high molecular weight plasmid. Also, that the antibiotic resistance is encoded on a high molecular weight plasmid, and can easily spread in the community thru food stuff generally consumed by the common man. Further studies need to be done on these high molecular weight R plasmids by performing restriction endonuclease digestion and gene sequencing.

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