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Research Communication

Hospital effluent: A source of multiple drug-resistant bacteria

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The present work was carried out to study the spread of multiple drug-resistant (MDR) bacteria from hospital effluent to the municipal sewage system. The MDR bacteria population in hospital effluents ranged from 0.58 to 40% for ten hospitals studied while it was less than 0.00002 to 0.025% for 11 sewage samples from the residential areas. Further, the MDR bacteria carried simultaneous resistance for most of the commonly used antibiotics and obviously the spread of such MDR bacteria to the community is a matter of grave concern.

DEVELOPMENT of drug resistance has followed the discovery of antimicrobial agents like a faithful shadow. Drug resistance observed till 1954 was through solitary events of bacterial chromosomal gene mutations. However,

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Shigella dysentery outbreak of 1954 in Japan was documented through bacteria simultaneously resistant to several antibiotics. Watanbe[1] showed that the multiple drug resistance (MDR) was through extrachromosomal autonomous genetic elements. He rightly referred to MDR as infectious drug resistance, since it could be transferred *en block* to sensitive bacteria during their cell to cell contact and subsequently these plasmids have been referred to as resistance plasmids (R-plasmids). Presently R-plasmid carrying bacteria are a major cause of hospital-borne infections. The antimicrobial selective pressure through indiscriminate use of antibiotics has played a significant role in enriching the MDR R+ strains in the hospital practice. The situation has reached such an ugly state largely in developing countries like India, that a sizeable number of hospital strains have become resistant simultaneously to most of the available antibiotics[2]. Hospitalized patients become heavily colonized with R+ strains mainly in their gut[3]. Infection with MDR bacteria may be transferred to other patients in the hospital resulting in cross-infections which are referred to as hospital-borne infections. The exact magnitude of hospital-borne infections is not precisely estimated for India but is expected to be around 10% and is much higher in intensive care units. Acquisition of MDR bacterial infections in hospitals may pose serious therapeutic difficulties.

The greatest fear was the transfer of resistance to pathogens like *S. typhi*, which 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[4].

The transfer of R-plasmids has been shown to occur in extra intestinal environment like the sewage system[5]. The dangers of infectious hospital waste received a great deal of attention in the last decade and a main emphasis on hospital solid waste; but liquid waste in the form of sewage has not received much attention. The present work was carried out to estimate the magnitude of MDR bacteria in hospital effluent and to compare it with the sewage from residential areas in city of Indore.

Three effluent water samples from each of the ten hospitals in Indore city were collected at 9 a.m., 2 p.m. and 6 p.m. from the outermost chambers before the drainage flows to the municipal sewage. From hospital No. 4, thirty samples of effluent were collected during the month. In addition, samples were collected 100 m and 2 km away from hospital No. 4 in the municipal sewage system. Triplicate samples from main chambers of sewage lines distributed in eleven major residential colonies of the city were also taken.

All the samples were subjected to viable count studies by spreading 100 µl of 10^{-1} to 10^{-4} dilution prepared in sterile saline over the nutrient agar plate. The plates were incubated overnight at 37°C and plates showing 50 to 200 colonies were used for expressing the total viable bacterial count.

The MDR problem encountered in hospital practice mainly involves Gram-negative bacteria. Hence for the estimation of the MDR bacteria, 100 µl diluted samples were spread over MacConkey agar plates supplemented with 30 µg/ml of chloramphenicol and 20 µg/ml of gentamicin. Chloramphenicol and gentamicin were selected because they represent two of the commonly used antibiotics over the last thirty years and also have greater *in vitro* stability. Differentiation as lactose fermenter and non-lactose fermenter could be made on Mackonkey agar for MDR isolates. A minimum of three colonies with similar morphology were selected individually and subjected to identification by standard biochemical methods and also subjected to drug susceptibility by the disk-diffusion technique of Bauer *et al.*[6].

The total viable bacterial and MDR bacterial counts (mean of three samples each) for hospital effluents and residential colony sewage samples are shown in Table 1. The per cent MDR bacteria population was significantly higher for hospital effluent samples than for the residential colony sewage samples ($P < 0.01$ by Student's t test). Another observation was the relatively higher total bacterial counts for sewage samples from residential colonies in contrast to hospital effluent samples. This could be due to greater usage of disinfectants and antibiotics in hospital

Table 1. Total and bacterial counts of domestic and hospital effluent samples

Source	Total count (CFU/ml) × 10^4	MDR (%)
Domestic		
D1	40 ± 7.07	Nil
D2	60000 ± 100000	Nil
D3	10 ± 4.27	Nil
D4	80 ± 20	Nil
D5	600000 ± 264575	0.0000011
D6	500000 ± 115470	0.000004
D7	500000 ± 100000	0.00002
D8	500000 ± 264570	0.00002
D9	25000 ± 13228	0.0004
D10	400 ± 142.42	0.0175
D11	400 ± 200	0.025
*Hospital**		
H1 (50)	60 ± 10	0.58
H2 (80)	80 ± 10	0.63
H3 (125)	8 ± 1.3	1.33
#H4 (400)	9 ± 0.72	1.5
H5 (700)	3 ± 0.866	2.88
H6 (200)	4 ± 1.802	3.57
H7 (50)	68 ± 31.432	4.41
H8 (50)	5000 ± 264	10
H9 (200)	20 ± 8.66	12.5
H10 (50)	5 ± 2.12	40

*Numbers in bracket indicate beds in the hospitals.
#Data are mean of 30 replicates.
Data are mean of three replicates ± standard deviation.
Total count expressed from number of colonies on nutrient agar while MDR expressed from number of colonies of Gram-negative MDR on antibiotic supplemented Mackonkey agar.

Table 2. Resistance patterns of MDR bacteria isolated from hospital effluents

Antibiotic group	Antibiotic/(conc.)*	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Penicillins	Ampicillin (10 µg)	R	R	R	R	R	R	R	R	R	R
	Piperacillin (100 µg)	R	R	R	R	R	R	R	R	R	R
Penicillin + β -lactamase inhibitor	Amoxycillin (20) + clavulinic acid (10 µg)	R	R	R	R	R	R	R	R	R	R
	Ticarcillin (75) + clavulinic acid (10 µg)	R	S	R	R	R	R	R	R	R	R
Cephalosporins	Cefoperazone (75 µg)	PR	R	R	R	R	R	R	R	R	R
	Ceftazidime (30 µg)	R	R	R	R	R	R	R	R	R	R
	Cefuroxime (30 µg)	R	R	R	R	R	R	R	R	R	R
	Cephaloridine (30 µg)	R	R	R	R	R	R	R	R	R	R
	Cefotaxime (30 µg)	R	R	R	R	R	R	R	R	R	R
Quinolones	Ciprofloxacin (5 µg)	R	R	R	R	R	R	R	R	R	R
Aminoglycoside	Amikacin (30 µg)	S	S	S	S	S	S	S	S	S	S
	Gentamicin (10 µg)	R	R	R	R	R	R	R	R	R	R
	Netilmycin (30 µg)	R	R	R	R	S	R	R	R	R	R
Others	Nitrofuran (300 µg)	S	S	PR	S	S	S	S	S	S	S
	Cotrimoxazole (23.75 + 1.25 µg)	R	R	R	R	R	R	R	R	R	R
	Chloramphenicol (30 µg)	R	R	R	R	R	R	R	R	R	R

R = Resistant; S = Sensitive; PR = Partially resistant.
*Drug concentration in µg/disc mentioned in parentheses.

practice. Hospital effluents from hospital No. 4 had 1.5% MDR bacteria population, while after getting mixed with the municipal sewage stream, the MDR bacteria population persisted to an extent of 0.5% up to a length of 100 m and downstream to a level of 0.06% at a distance of 2 km. This clearly shows a higher influx and persistence of MDR bacteria from hospital effluents to the municipal sewage system.

The per cent MDR bacteria for hospital samples ranged widely from 0.58 to 40%, while for residential colony sewage it ranged between less than 0.00002 and 0.025%. A very high percentage of MDR in some of the hospitals could be due to excessive use of antibiotics resulting in increased selective pressure and in turn increase in the prevalence of MDR bacteria. Low loads of liquid waste generated due to scarcity of water in some of the hospitals might have as well given low dilution effect leading to an apparent rise in MDR bacteria population in the effluent.

The method of estimation of MDR bacteria clones in the effluent samples used in this study may have some limitations. The bacterial strains susceptible to gentamicin and chloramphenicol but resistant to other antibiotics must have been missed out during the estimation of MDR bacteria. Yet, the number of MDR bacteria was alarmingly high for the effluent samples from hospitals. More perturbing was the pattern of MDR (Table 2). Simultaneous resistance for ampicillin, amoxacillin + clavulinic acid, piperacillin, second and third generation cephalosporins, cotrimoxazole, gentamicin, netilmycin and quinolones like ciprofloxacin formed the common MDR pattern. The pattern was almost the same for the diverse species (*E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas*) grown from the effluent samples and strongly suggests prevalence of similar R-plasmids. The MDR pattern seen in the bacterial isolates from hospital effluent samples included most of the antibiotics used presently for treating human infections. The worst fear apprehended is the transfer of such resistance to bacterial pathogens causing infections in the community. In that case most of the presently available antibiotics will be futile against the infectious organisms. The origin of such MDR bacterial strains appears to be the hospital environment and the selective pressure responsible for expanding such bacterial populations in hospitals must have been through the use of drugs in humans and not from their use in the veterinary field or agriculture as pointed out by Walton[7].

The present observations suggest that hospital effluents can be a potential health hazard by adding MDR bacteria to a city sewage pool. Similar studies need to be carried out in other cities to tackle the obnoxious problem of MDR being passed on from hospitals to the community.

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