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The Occurrence of Antibiotic Resistant Salmonellas in Sewage and the Effect of Primary Sedimentation on their Numbers.

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RINGKASAN

Penggunaan antibiotik secara meluas telah mengakibatkan penyebaran strain-strain bakteria yang berintangan kepada antibiotik yang berkenaan dalam kumbahan dan perairan alam sekitar. Kajian ini telah menunjukkan bahawa bilangan Salmonella yang berintangan-antibiotik dengan yang sensitif tidak mempunyai perbedzaan bererti sebelum dan sesudah sedimantasi awal. Ini bermakna yang organisma-organisma itu telah mendapat kuasa rintangan ini daripada penggunaan antibiotik dan bukan semasa pengolahan kumbahan. Sedimentasi awal sahaja boleh mengurangkan lebih dari 80% daripada semua salmonella yang terdapat dalam kumbahan tetapi pengurangan ringan akan berlaku jika proses ini tidak dioptimumkan. Cara yang terbaik untuk mengawal penyebaran salmonella yang berintangan-antibiotik ialah dengan mengawal penggunaan antibiotik.

SUMMARY

The widespread use of antibiotics has led to the occurrence of resistant strains of bacteria in sewage and in the aquatic environment. This study has shown that there is no significant change in the proportion of antibiotic-resistant and antibiotic sensitive salmonellas during sedimentation of sewage and hence these organisms must have acquired resistance during the initial use of the antibiotic and not during sewage treatment. Primary sedimentation alone can remove more than 80% of the total salmonellas present in raw sewage but negligible reductions will occur if the process is not optimised. The best way of controlling the release of antibiotic resistant salmonellas into the environment is to control the prescription and use of antibiotics.

INTRODUCTION

The increasing use of antimicrobial drugs for the treatment of diseases in animals and humans, and for controlling infection in intensively-reared livestock by medication of food and drinking water, has led to the emergence of resistant strains of bacteria. Many strains of gram-negative organisms, particularly in the family *Enterobacteriaceae,* are capable of acquiring antibiotic resistance. This acquisition may occur either by genetic mutation or by the transfer from donor cells of bacteria possessing the so-called R^-factor (R^+strains) to recipient bacterial cells (R^-strains) which may be of the same species as the donor or a related species. The R^-factor is an extrachromosomal element of DNA, termed plasmid, which can replicate autonomously during cell division and which includes genetic factors conferring resistance to one or more antibiotics. It can be transferred during conjugation – a mating process which occurs between pairs of bacteria. Thus, for example, single or multiple drug resistance can be conferred by R^+ strains of *Escherichia coli* to other strains of *E. coli,* some of which are pathogenic, or to other enteric pathogens, such as *Salmonella.*

Earlier surveys (Neu *et al.,* 1975; Nakaya *et al.,* 1975) have demonstrated an increase in the proportion of *salmonellas* isolated from both humans and animals which are antibiotic resistant. They have a competitive advantage in terms of growth over similar strains of antibiotic sensitive bacteria in the presence of the relevant antibiotic. The establishment of resistant strains in any one environment e.g. in antibiotic treated drinking water, could increase the chances of infection since the antibiotics would reduce the competition from sensitive bacteria normally present in the water and in the digestive tract. Also if disease occurs, adequate treatment requires antibiotic sensitivity testing and the use of antibiotics to which the organism is susceptible. It is often a feature of transferable resistance that multiple resistance is carried, so that an increased hazard is presented to the patient.

This study examines the resistance to antibiotics of *Salmonella* isolates from three sewage treatment plants near Kuala Lumpur, Malaysia; the effect of primary sedimentation on their removal, and the probability of R-factor transfer in sewage.

MATERIALS AND METHODS

Samples

Raw and settled (after primary sedimentation) sewage were obtained from three geographically distinct locations in Kuala Lumpur. Plant A is a complete activated sludge system while treatment at Plant B consists of primary sedimentation only. The effluent from location C was from an overflow channel linked to a septic tank system. The settled sewage at both plants A and B was taken about 4 h after the raw sewage samples to allow for hydraulic retention through the sedimentation tanks. This represented the mean residence time for the sewage in the tanks. All samples were obtained near some points of turbulence to ensure a well-mixed representative sample. They were collected at weekly intervals over a period of a month.

Isolation of salmonellas

All samples were examined for salmonellas by enrichment in double strength Rappaport broth (Rappaport *et al.,* 1956). Enumeration for salmonellas was carried out according to the Most Probable Number (MPN) method as follows: five replicates each of a 1.0 ml, 5.0 ml and 10.0 ml raw and settled sewage samples. Septic tank effluents were pipetted into sterile universal bottles and an equal amount of Rappaport broth was added. The bottles were then capped and incubated in a water bath at 39°C for 24-36 h. After this time, a loopful of sample from each bottle was carefully streaked onto separate plates of XLD agar (OXOID) for incubation at 37°C for 24 h. Approximately 50 presumptive *Salmonella* colonies were isolated from each positive sample and streaked on nutrient agar (OXOID) to establish pure cultures. They were confirmed as salmonellas by agglutination in polyvalent-O salmonella antiserum (Wellcome Laboratories Ltd.) as well as by fermentation of sucrose, lactose, mannitol and dulcitol.

Antibiotic resistance test

All the Salmonella isolates were examined for their resistance to eight antibiotics. Oxoid multodisks (OXOID) were aseptically placed on a lawn of each of the isolates on nutrient agar. The plates were then incubated at 37°C for 24 h.

RESULTS

The results in Table 1 show a marked difference in the *Salmonella* removal efficiencies during primary sedimentation at plants A and B. Although both plants have similar sedimentation tanks, those at plant B were more efficient despite the influx of larger volumes of waste water. The less efficient performance of the tanks at plant A is probably due to short circuiting of flow through the tanks resulting in a reduced hydraulic retention time. In addition, the surfaces of the tanks were not level due to a geographical tilt in the land area. The results from plant B are in close agreement with other reports in the literature concerning sedimentation tank efficiencies in both tropical and temperature climates (Mom and Schaeffer, 1940; Yaziz and Lloyd, 1979).

TABLE 1
Salmonella removal during primary sedimentation

Plant	No. of salmonellas per liter**				Percentage removal
	Raw sewage	S.D.††	Settled sewage	S.D.	
A	3,500	2484	1,600	746.2	54.30
B	5,500	5419	1,000	340.0	81.80
*C	–	–	2,250	786.9	–

* Septic tank
** Median of four values
†† Standard Deviation of populations

The data in Table 2 indicate the percentage of isolates resistant to the specific antibiotics tested. Examination of the combined data using the Wilcoxon two-sample test showed no significant difference between the proportions of antibiotic resistant salmonellas found in samples of raw sewage and settled sewage. There was also no significant difference between the raw and settled sewage in resistance to each of the antibiotics tested (chi-squared test, $p < 0.01$).

The number of strains resistant to one or more of the antibiotics tested was generally low, ranging from 2% to 17% of the total number of isolates. Ampicillin resistant strains reached 40.40% but this is still not high compared with those reported for salmonellas isolated from

TABLE 2

The percentage of *Salmonella* isolates from sewage and sewage effluents showing resistance to various antibiotics.

Location	Sample (number of isolates)	Antibiotics*							
		N	K	Ch	P	PN	SCT	S	Te
Plant A	Raw sewage (50)	0	2.0	4.0	100.0	8.0	0	0	0
	Settled sewage (50)	0	0	2.0	100.0	6.0	0	0	0
Plant B	Raw sewage (47)	0	0	14.8	100.0	40.4	17.0	8.5	4.3
	Settled sewage (44)	4.2	0	9.0	100.0	22.7	4.2	4.2	0
Site C	Septic tank effluent (48)	4.2	0	2.0	100.0	75.0	0	0	0

* N, neomycin; K, kanamycin; Ch, chloramphenicol; P, penicillin G; PN, ampicillin; SCT, sulphamethoxazole-trimethoprim; S, streptomycin; Te, tetracycline.

humans and animals in other countries (Anderson, 1968; Pocurull *et al.,* 1971; Wiedemann and Knothe, 1971). The resistance of all the isolates to penicillin G conformed to expectations as this antibiotic is primarily directed against the Gram-positive bacteria.

DISCUSSION AND CONCLUSION

Fontaine and Hoadley (1976) (Table 3) reported that the frequency of isolates of the same species resistant to one or more antibiotics was substantially reduced after sewage treatment. However, in this study involving primary sedimentation only, there is no suggestion of the trend found by these workers. Although a marked reduction was observed in the total number of salmonellas after primary sedimentation, there was no significant difference in the ratio of antibiotic-resistant to antibiotic-sensitive salmonellas between the raw and the corresponding settled sewage. The considerable variation in the proportion of strains of salmonellas in sewage resistant to a given antibiotic, which is most marked in samples taken from plants A and B, reflects use of antibiotics locally, which will influence the pattern of resistance of organisms excreted. Linton (1977) observed this effect with animal herds receiving short-term antibiotic therapy.

This study has shown that there is no significant increase in the proportion of antibiotic resistant salmonellas in the settled sewage after primary sedimentation. This suggests that the antibiotic resistant salmonellas in the settled

TABLE 3

The percentage of salmonellas and coliform bacteria isolated form sewage and faeces resistant to certain antibiotics (summarised from References indicated)

Reference	Sample (number of strains)	Antibiotic							
		N	K	Ch	Ce*	PN	Su*	S	Te
Nakaya *et al.* (1975)	Human clinical specimens – (Salmonellas) (1,151)	–	3.04	2.25	–	2.25	–	85.0	29.5
Fontaine, T.D. & Hoadley, A.W., (1976)	Sewage (33) (Coliforms)	66.7	66.7	0	6.1	9.1	12.2	63.6	21.2
	Effluent (29) (Coliforms)	6.9	6.9	0	27.6	6.9	12.2	24.1	27.6
Carrington, E.G.	Sewage (46) (Coliforms)	–	–	8.7	–	52.2	–	23.9	8.7
	Effluent (47) (Coliforms)	–	–	4.2	–	82.9	–	4.3	2.1

* Ce, cephalothin; Su, sulphatiazole.

sewage acquire resistance as a result of antibiotic use and not from the transfer of R^+ factors from donor strains while in the sedimentation tanks. A similar observation was also made by Carrington (1979) for the resistance of coliforms to the antibiotics listed in Table 3 during sewage treatment.

In a satisfactorily operating sedimentation tank, the number of salmonellas would be reduced by more than 80%. Where this is the only method of treatment, as in plant B or where the sedimentation process is not at its optimum, a substantial number of the antibiotic resistant salmonellas will be released into the environment. Whether this represents a potential threat to public health or not cannot be clearly ascertained and it remains to be proven that the antibiotic resistant strains are able to survive for longer periods in the aquatic environment than susceptible strains, except in the presence of the relevant antibiotic. The widespread use of antibiotics in treatment of disease and for the medication of food and water in intensive livestock farming are the main reasons for the occurrence of these resistant bacteria in sewage. Their numbers in the environment may be reduced by controlling or restricting the prescription and use of antibiotics.

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