Instability and linkage of silver resistance, lactose fermentation, and colony structure in *Enterobacter cloacae* from burn wounds

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SYNOPSIS A strain of *Enterobacter cloacae* from a burn wound has displayed unstable resistance to silver nitrate and a linkage of this characteristic to that for rapid lactose fermentation and mucoid colony structure. Attempts to demonstrate transferability of these unstable determinants gave negative results.

The use of silver preparations for topical antimicrobial therapy in the Burns Unit of this Hospital has prompted routine screening of bacterial isolates for resistance to this metal.

During some early tests in this project two strains of *Enterobacter cloacae* were isolated which displayed high but unstable resistance to silver nitrate. Each was isolated from a different patient in the Burns Unit.

The two strains differed in that in one of them (Z173) silver resistance was linked to rapid lactose fermentation and to mucoid colony structure. In the other (Z147), the progeny were homogeneously slow lactose fermenters and yielded non-mucoid colonies. These features seemed of sufficient interest to warrant being recorded and the present paper is concerned mainly with the evidence for the instability and linkage of the characters in Z173.

Methods

Cultures were grown in nutrient broth (Oxoid No. 2) and colonies were screened for lactose fermentation on MacConkey agar (Oxoid No. 2). Screening of colonies for resistance to silver nitrate (40 µg/ml) and measurement of minimum inhibitory concentrations of the compound was done with a salt-free agar medium (McHugh *et al.*, 1975).

Experiments to detect transfer of silver resistance were conducted in Brain Heart Infusion Broth (Difco) using as a recipient a strain of *Escherichia coli* (M2243) which was ONPG negative, resistant to

nalidixic acid, and auxotrophic for histidine and several other amino acids.

Organisms were preserved by drying and were stored in air for short-term and *in vacuo* for long-term maintenance (Annear and Grubb, 1973).

Results

A colony of Z173 was checked for silver resistance, cultured in broth, and spread on a MacConkey plate which was incubated at 30°C for 20 hours. The majority of the resulting colonies were bright red, indicating rapid lactose fermentation, and were mucoid in texture; the remainder were pale pink, non-mucoid, and smaller than the rapid lactose fermenters. The pale pink colonies assumed a deeper red after several days of further incubation and were shown to be ONPG positive as were the rapid fermenters. Single colonies of the rapid fermenters were transferred to tubes of broth which were cultured at 30°C. The cultures were sampled periodically and spread for the single colonies on MacConkey plates. During a period of 10 days the fraction of rapid lactose fermenters fell from a level of approximately 90% to one of <1%.

Screening of the colonies on silver plates by multi-point replication (Annear and Grubb, 1972) during these experiments revealed a firm linkage of silver resistance with rapid lactose fermentation and mucoid colony structure. No exceptions were found in tests with 500 colonies of each category of lactose fermenter.

The minimum inhibitory concentration of silver nitrate for the rapid lactose fermenters was >1000

$\mu\text{g/ml}$ whereas that for the slow lactose fermenters was approximately $25 \mu\text{g/ml}$. The values were obtained for both heavy and light inocula (10^6 and 10^2 organisms respectively).

When silver sensitive, slow lactose fermenting variants were grown in broth and stored for several weeks at room temperature, colony screening revealed no reversion to either silver resistance or rapid lactose fermentation.

In attempts to transfer silver resistance, a cross was set up using log phase cultures of the donor and the recipient in a ratio of approximately 1:100. After 20 hours at 37°C the mating mixture and appropriate controls were spread over salt-free MacConkey medium containing nalidixic acid ($50 \mu\text{g/ml}$) and silver nitrate ($50 \mu\text{g/ml}$). No transfer of either silver resistance or rapid lactose fermentation was obtained.

Discussion

The high frequency with which the linked characteristics of silver resistance and rapid lactose fermentation are lost in broth cultures of Z173 is strong evidence for an extrachromosomal (plasmid) location of the determinants concerned. The mucoid colony structure which accompanies these features is probably not governed by a separate determinant but is the consequence of the active lactose metabolism.

Although these determinants are spontaneously and rapidly lost during storage in broth culture, they are apparently not transmissible and are thus of a non-conjugative type. Since both parent and variant cells are ONPG positive and thus contain β -galactosidase which attacks lactose, it seems likely that the variants are lacking the permease activity which governs the entry of the carbohydrate into the cells. A 'cryptic' mutant of *E. coli* showing this property was reported by Herzenberg (1959) in his comprehensive study of the subject.

Silver resistance has been described previously in bacteria isolated from patients in other Burns Units. Rosenkranz *et al* (1974) reported it in isolates of *Enterobacter cloacae* where resistance to silver was manifested when the metal was tested as silver sulphadiazine or as silver benzoate but not as silver nitrate. Attempts to demonstrate transferable resistance in these strains gave negative results. McHugh *et al* (1975) discovered resistance to silver nitrate in isolates of *Salmonella typhimurium* and demonstrated the determinant together with those for resistance to a number of other metals and antibiotics to be transferable to sensitive recipients of *E. coli* and *S. typhimurium*. Neither group of these workers reported on tests for instability of the resistance determinants.

Williams-Smith and Parsell (1975) have recently reviewed work concerning the transmissibility determinants for substrate utilization and other biochemical activities used to differentiate bacterial genera and species. The present study has shown the likelihood of the permease for lactose fermentation in Z173 to be extrachromosomal and unstable but not apparently transmissible.

The close biochemical similarity between Z173 and Z147 and their recovery from patients in the same area of the hospital suggest that the isolates are genetically related. It is possible that a dissociation may have occurred in the usually firm linkage between the determinants for silver resistance and lactose fermentation, thus yielding a slow lactose fermenter with unstable silver resistance. Further sampling of specimens for these organisms may have been rewarding in this respect.

Although a considerable amount of work has been done on the transmissibility and instability of genetic determinants in Gram-negative bacilli it appears that these two phenomena are rarely studied in parallel and there is a need for examination of the relationship between them. Studies in progress with the present isolates are being directed along these channels and include further work with antibiotic resistance determinants which they harbour but which have not been reported here. Some preliminary experiments have shown that these determinants, unlike that for silver resistance, are readily transmissible.

In view of the work of Rosenkranz *et al* (1974) it would be of interest to carry out studies on the resistance of the present isolates to other silver compounds particularly silver sulphadiazine which is used in this hospital and also widely elsewhere for the topical treatment of bacterial infection.

The clinical significance of the findings presented here is yet to be elucidated. However, it seems that while the use of silver compounds may well result in the selection of silver resistant organisms, spontaneous loss of such resistance occurs readily in the absence of the selective pressures. As yet this has been observed only *in vitro* but may possibly occur also in *in vivo*.

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