Antiseptic and antibiotic resistance in Gram-negative bacteria causing urinary tract infection

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SUMMARY A collection of 802 isolates of Gram-negative bacteria causing urinary tract infections was made from general practice, antenatal clinics, and local hospitals. The organisms were tested for their sensitivity to chlorhexidine, cetrimide, glutaraldehyde, phenyl mercuric nitrate, a phenolic formulation, and a proprietary antiseptic containing a mixture of picloxidine, octyl phenoxy polyethoxyethanol, and benzalkonium chloride. Escherichia coli, the major species isolated, proved to be uniformly sensitive to these agents. Approximately 10% of the total number of isolates, however, exhibited a degree of resistance to the cationic agents. These resistant organisms were members of the genera Proteus, Providencia, and Pseudomonas; they were also generally resistant to five, six, or seven antibiotics. It is proposed therefore that an antiseptic policy which involves the intensive use of cationic antiseptics might lead to the selection of a flora of notoriously drug-resistant species.

During the course of studies on the development of urinary tract infection in patients undergoing intermittent bladder catheterisation in the early stages of paraplegia, observations were made on the effect of the repeated application of the antiseptic chlorhexidine on the bacterial flora of the urethral meatus. The bladder management of these patients involved the washing of the penis with aqueous solutions of chlorhexidine (600 µg/ml) before the insertion of the catheter at approximately 8-hour intervals. The flora was examined daily before and after application of the antiseptic, and the general pattern that emerged from this work was that for the first few days after trauma the meatus carried a Gram-positive flora which the chlorhexidine usually eliminated. A Gram-negative population developed subsequently, however, and proved to be more refractory to chlorhexidine. In particular, Proteus mirabilis, Pseudomonas aeruginosa, Providencia stuartii, and Klebsiella frequently survived the application of the antiseptic, and in laboratory tests many of the strains demonstrated an ability to grow in media containing 200 µg/ml of the agent. Subsequently, some of the Pr. mirabilis and P. stuartii strains from this source were shown to have minimum inhibitory concentrations (MICs) of up to 800 µg/ml chlorhexidine, well above the level of 10-50 µg/ml originally reported to inhibit the growth of Gram-negative species.

These chlorhexidine-resistant strains were isolated from an unusual clinical situation in which repeated exposure to the antiseptic over periods of up to 3-4 weeks takes place. It is perhaps understandable that resistant strains should be isolated from a situation where such a strong selective pressure is operating. However, as resistance to agents like chlorhexidine is not generally screened for in clinical laboratories it is not possible to say whether resistance to antiseptics is limited to such special circumstances or is a more general phenomenon. The objective of this study was to observe the sensitivity of Gram-negative organisms causing urinary tract infections, in a variety of types of patients, to a number of antiseptics and disinfectants. A collection of organisms was therefore made from general practice, antenatal clinics, and inpatients at five local hospitals. The sensitivity of these isolates to chlorhexidine, cetrimide, glutaraldehyde, phenyl mercuric nitrate, a phenolic formulation, and a proprietary antiseptic containing a mixture of picloxidine, octylphenoxy polyethoxyethanol, and benzalkonium chloride was determined. In addition, the antibiotic resistance patterns were recorded to

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explore any possible relationship between disinfectant and drug resistance.

Methods and material

BACTERIAL STRAINS
The clinical isolates used in this study all originated from cases of significant bacteriuria (> 10^9 viable cells/ml urine). They were obtained over a 12-month period from a hospital laboratory which served several hospitals and local health centres in the Cardiff area. To avoid duplication, repeated isolates of the same species from individual patients were excluded from the survey. The organisms were identified using the methods of Cowan and Steel and in some cases with the API 20E Microtube System (API Products Ltd). Escherichia coli NCTC 10418 and Pr. mirabilis 619 were used as control organisms in the sensitivity testing.

ANTIBACTERIAL AGENTS
The chlorhexidine digluconate and the cetrimide were obtained from ICI Ltd. Glutaraldehyde was purchased from Sigma Ltd and phenyl mercuric nitrate from BDH Ltd. The phenolic preparation contained 16% v/v of a mixture of chloroxylenol, chlorocresol, chlorophene, sodium O-phenylphenate, and sodium pentachlorophenate in a basis of anionic emulsifier and alcohol (Hycolin, Pearson Ltd, Clough Road, Hull). The sixth antibacterial agent was the proprietary antiseptic Resiguard (Nicholas Laboratories Ltd) which contains picloxidine digluconate (1% w/v), octyl phenoxy polyethoxyethanol (11-0% w/v), and benzalkonium chloride (12% w/v).

MIC DETERMINATIONS
As the inoculum history of bacterial cultures used in antimicrobial sensitivity tests may affect the results obtained a standard procedure was adopted for testing the clinical isolates. A single colony from a pure culture of each strain growing on MacConkey agar after overnight incubation at 37°C was inoculated into nutrient broth (Oxoid Nutrient Broth No. 2). After 18 hours incubation at 37°C, volumes (5 μl) of a 10^-4 dilution of the broth culture, each containing approximately 10^8 viable cells, were dropped on to the surface of nutrient agar plates containing various concentrations of the antibacterials. A standard method was adopted for the preparation of the plates. The agents were added to molten agar that had been allowed to cool to 50°C, and the plates were poured immediately. When set, the plates were dried for 20 minutes at 37°C and then used directly.

The lowest concentration of the agent preventing colony formation after overnight incubation at 37°C was taken as the MIC for that strain. The tests were routinely performed in duplicate and, with each batch of strains tested, E. coli 10418 and Pr. mirabilis 619 were included as control reference organisms with known MICs.

ANTIBIOTIC SENSITIVITY
The antibacterial sensitivity patterns of the isolates were determined by seeding plates of DST agar (Oxoid Ltd) with young log phase broth cultures (0.1 ml) and applying U3 Multodiscs (Oxoid Ltd), which are impregnated with the following antibiotics: gentamicin (10 μg), colistin (10 μg), nitrofurantoin (200 μg), sulphafurazole (500 μg), kanamycin (30 μg), ampicillin (25 μg), sulphonmethoxazole/trimetprim (25 μg), and tetracycline (50 μg). E. coli NCTC 10418 was used as sensitive control with each batch of strains tests.

Results

In a 12-month period, over 1000 isolates of Gram-negative bacteria were obtained from urinary infections. E. coli proved to be by far the most prevalent species, and after six months when some 369 E. coli isolates had been investigated, the sensitivity testing of this species was discontinued. The species distribution of the isolates examined in the survey is shown in Table 1. The results of the sensitivities of these organisms to the six antibacterials are summarised in Figures 1 to 6. The histograms represent the percentage of isolates having each MIC, grouped on the basis of the multiplicity of their antibiotic resistance. It can be seen that E. coli was uniformly sensitive to the antibacterials, having MICs of the six agents equivalent to that of the control E. coli strain 10418 and well below the recommended use concentrations of these agents. The situation is different with Proteus, P. stuartii,

Table 1 Bacterial species isolated from urinary tract infections over a 12-month period

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>369*</td>
</tr>
<tr>
<td>Pr. mirabilis</td>
<td>139</td>
</tr>
<tr>
<td>Pr. morganii</td>
<td>31</td>
</tr>
<tr>
<td>Pr. vulgaris</td>
<td>10</td>
</tr>
<tr>
<td>Pr. retgeri</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>167</td>
</tr>
<tr>
<td>P. stuartii</td>
<td>24</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>35</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>12</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>9</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>3</td>
</tr>
<tr>
<td>Serratia marcesens</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>802</strong></td>
</tr>
</tbody>
</table>

*Number of isolates obtained in first six months of the study.
and *Pseudomonas*, where MICs substantially higher than those of the control strain were recorded for the cationic antiseptics.

In deciding whether strains are resistant or sensitive to antiseptics or disinfectants, it is difficult to know what criterion to apply. With antibiotics it is usual to designate organisms as resistant if they are able to multiply in the maximum concentration of the drug attainable at the site of the infection. This sort of yardstick is clearly not applicable where antiseptics and disinfectants are concerned. For the purpose of this study it was decided that if the MIC value for an organism was greater than the concentration of the agent normally recommended for routine use, then that isolate was designated as resistant. When this criterion is applied (Table 2) it appears that all the isolates were sensitive to glutaraldehyde, the phenolic mixture, and phenylmercuric nitrate. A small percentage of the total were resistant to the cationic agents.
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Fig. 2 Sensitivity of the isolates to cetrimide

Table 2 Percentage of isolates of each species with MIC values greater than the normal recommended use concentration of each antibacterial

<table>
<thead>
<tr>
<th>Organism</th>
<th>Chlorhexidine MIC &gt; 500 μg/ml</th>
<th>Cetrimide MIC &gt; 1000 μg/ml</th>
<th>Picloxidine mixture MIC &gt; 0.625 μg/ml</th>
<th>Glutaraldehyde MIC &gt; 2% v/v</th>
<th>Phenolic mixture MIC &gt; 2% v/v</th>
<th>Phenyl mercuric nitrate MIC &gt; 20 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>1.8</td>
<td>0.0</td>
<td>0.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Proteus</td>
<td>38.7</td>
<td>2.2</td>
<td>35.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>P. stuartii</td>
<td>83.3</td>
<td>29.2</td>
<td>75.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>45.7</td>
<td>65.7</td>
<td>60.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Others</td>
<td>3.8</td>
<td>3.8</td>
<td>7.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>% of total No. of isolates</td>
<td>13.7</td>
<td>4.4</td>
<td>13.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
A comparison between the chlorhexidine sensitivity of isolates from hospital inpatients and those from general practice and antenatal clinics is shown in Figure 7. It is clear that resistant organisms are mainly found in infections originating in hospital.

**Discussion**

The variety of Gram-negative species isolated and tested in this survey reflects the range of organisms that have been reported to be responsible for acute, chronic, and hospital-acquired urinary infection. The most common organism, *E. coli*, proved to be extremely sensitive to all the antibacterials, the MIC values recorded for the isolates being equivalent to that of the control culture collection strain *E. coli* 10418 and well below the normally recommended use concentrations for the six agents.

The criterion used to describe strains as resistant was an MIC greater than the normally used con-
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Fig. 4  Sensitivity of the isolates to the phenolic agent.

Although it was fully realised that the MIC value for a disinfectant may not represent the concentration that will be effective in practice, some support for the relevance of such a standard was provided by Thomas et al.\textsuperscript{11} who showed that urine-grown cultures of organisms with MICs of chlorhexidine > 800 μg/ml survived well when challenged with the recommended level of this antiseptic. When the criterion was applied to the results of this survey it can be seen that the resistance was limited to the cationic agents and to the genera Proteus, Providencia, and Pseudomonas. There have been previous reports of this phenomenon. Chlorhexidine resistance has been reported in a Pseudomonas sp.\textsuperscript{12}, Ps. aeruginosa,\textsuperscript{13} Pr. mirabilis,\textsuperscript{13,14} and P. stuartii.\textsuperscript{4} In all these instances, the organisms were isolated from sources where they had been exposed to the antiseptic. In the present survey, Fig. 7 shows that the resistant strains came predominantly from inpatient infections. In fact, the majority came...
from chronic infections in geriatric or paraplegic patients. Infections in these cases are often with mixed populations, and the observation that the antiseptic-resistant organisms were also generally resistant to five, six, or seven antibiotics (Figs 1-3) leads to speculation that the challenge of such a mixed culture with, for example, an instillation of chlorhexidine into the bladder might result in the selection of the chlorhexidine-resistant, drug-resistant species.

The general conclusion from the survey is that resistance to antiseptics and disinfectants is not a widespread phenomenon in organisms that are responsible for urinary tract infections. Resistance appears to be limited to the genera *Pseudomonas*, *Proteus*, and *Providencia* and to the cationic antiseptics. The resistant strains in this survey were mostly isolated from patients in whom chlorhexidine was being used to wash down the urethral meatus before insertion of a bladder catheter. A chlorhexi-
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MIC of E. coli 10418

-0.2 µg/ml

Multiplicity of antibiotic resistance

0 1 2 3 4 5 6 7

% of isolates with each MIC

≤0.1 0.2 0.5 1.0 2.0 4.0

PMN (µg/ml)

Fig. 6 Sensitivity of the isolates to phenyl mercuric nitrate (PMN).

Dine-containing cream was used to facilitate the passage of the catheter through the urethra, and in some cases the antiseptic was incorporated into the urine drainage bag. These observations, together with an examination of the antibiotic resistance patterns of the isolates, have led us to the conclusion that an antiseptic policy which relies on the intensive use of cationic antiseptics such as chlorhexidine could well result in the selection of a flora of notoriously drug-resistant species.

The recent report that resistance to the antiseptic hexachlorophane in Ps. aeruginosa was determined by an extrachromosomal element which also carried the genes for sulphonamide and gentamicin resistance has important implications and has led us to consider whether the observed correlation between antibiotic and cationic antiseptic resistance has a basis in an association of the resistance genes. The possibility of such a relationship existing between the genetic factors for chlor-
hexidine and antibiotic resistance in *P. stuartii* is now under investigation in this laboratory.

References


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