ANTIBIOTIC SUSCEPTIBILITIES OF ACHROMOBACTER XYLOSOXIDANS

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SYNOPSIS

Antibiotic susceptibilities of 25 strains of *Achromobacter xylosoxidans* to 27 antimicrobial agents were determined. These organisms were fairly uniform in their susceptibility to B-lactam antibiotics and resistance to aminoglycosides. Azlocillin, moxalactam and piperacillin were the three most active drugs, inhibiting all strains at 2 mg/L or less. Cefotaxime and chloramphenicol were moderately active. Most of the strains were multiresistant to 13 to 15 drugs each, including six aminoglycosides, penicillin, cefuroxime, tetracycline, erythromycin, colistin, rifampicin and nitrofurantoin. Strains isolated from hospital inpatients were not very different in their antibiotic susceptibilities from those isolated from community-acquired sepsis.

INTRODUCTION

Achromobacter xylosoxidans, a non-fermentative Gram-negative bacillus has been associated with a variety of clinical conditions ranging from community-acquired superficial sepsis (1) to potentially fatal nosocomial infections (2, 3). We have described in a separate article the bacteriology and clinical significance of 30 strains recovered from patients and fomites. In this paper, we report the results of antibiotic susceptibility tests for 25 of these strains.

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MATERIALS AND METHODS

Organisms

The twenty five strains of *A. xylosoxidans* examined consisted of 16 strains from hospital inpatients, seven strains from outpatients and two strains from fomites. The identity of all these strains were confirmed by the Computer Trials Laboratory, National Collection of Type Cultures, Central Public Health Laboratory, Colindale, London. Isolates were kept on nutrient agar slopes and lyophilized within seven to ten days of isolation.

Antibiotics

Stock solutions of antibiotic powders obtained from various manufacturers were kept at -20°C until required for sensitivity testing. The sources of antibiotics were: Sigma (ampicillin, streptomycin, gentamicin, amikacin, trimethoprim, chloramphenicol, colistin); Beecham Pharmaceuticals (carbenicillin, ticarcillin); Eli Lilly (moxalactam, tobramycin); Glaxo (cefuroxime, ceftazidime); Roche (ceftriaxone, sulphamethoxazole); Meiji Seika (kanamycin); Lederle (piperacillin); Pfizer (cefoperazone); Bayer (azlocillin); Schering (netilmicin) and Hoechst (cefotaxime).

Disc diffusion tests

Test organisms were inoculated onto Iso-sensitest agar (Oxoid) to give semi-confluent growths. Antibiotic discs (Mast Laboratories) were applied onto the surface of inoculated plates which were then incubated aerobically for 18-24 hours. A control plate of *Escherichia coli* NCTC 10418 was put up with each test. Inhibition zone diameters were measured for test and control organisms and recorded as sensitive, intermediate or resistant after comparing test organisms with the control.

Minimum Inhibitory Concentrations (MICs)

MICs were determined using the agar plate dilution method. Two-fold antibiotic dilutions were made in Mueller-Hinton agar (Difco Laboratories). Strains of *A. xylosoxidans* were grown on nutrient agar overnight and suspended in nutrient broth to a turbidity equivalent to 0.5 McFarland standard barium sulphate solution, using a spectrophotometer. The suspensions were further diluted 1 in 100 and inoculated onto antibiotic plates by a Denley multipoint inoculator. The plates were then incubated overnight at 36°C. The lowest antibiotic concentration that permitted no growth of the organism was considered the MIC. *Escherichia coli* NCTC 10418 and Oxford *Staphylococcus aureus* NCTC 6571 were used as control organisms. Iso-sensitest agar (Oxoid) was used instead of Mueller-Hinton agar for the determination of trimethoprim and sulphamethoxazole MICs

RESULTS

Table 1 shows the results of MIC determinations for 20 antimicrobials. Table 2 summarizes those results

TABLE 1
MINIMUM INHIBITORY CONCENTRATIONS OF 25 STRAINS OF *A. XYLOSOXIDANS*

	MIC (mg/L)									
	Cumulative %									
Antibiotic	**≤ 0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**≥ 128**
Ampicillin			80	96	96	100				
Acarbenicillin		24	52	52	88	92	96	100		
Piperacillin	40	76	96	100						
Azlocillin	68	80	100							
Ticarcillin	28	48	64	92	96	96	100			
Moxalactam		92	96	100						
Cefuroxime			0	4	4	4	4	4	4	100
Cefotaxime				0	4	20	60	80	100	
Cefoperazone		32	52	88	100					
Ceftazidime	8	8	44	72	100					
Ceftriaxone	4	8	8	8	24	40	64	76	100	
Streptomycin									0	100
Kanamycin				0	4	4	4	16	48	100
Gentamicin						0	12	32	40	100
Amikacin					0	4	0	0	28	100
Tobramycin					0	16	28	52	60	100
Netilmicin						0	16	28	40	100
Trimethoprim/ Sulphamethoxazole*	64	76	76	76	76	76				
Chloramphenicol					0	20	80	100		
Colistin				0	4	8	16	40	48	100

*Trimethoprim and sulphamethoxazole tested at a 1:19 ratio from 0.03 mg/L of trimethoprim and 0.6 mg/L of sulphamethoxazole to 8 mg/L of trimethoprim and 152 mg/L of sulphamethoxazole.

TABLE 2
SUSCEPTIBILITY OF 25 STRAINS OF *A. XYLOSOXIDANS* TO 27 ANTIBIOTICS

Antibiotic	% strains		
	Sensitive	Intermediate	Resistant
Ampicillin*	100	0	0
Carbenicillin	96	4	0
Piperacillin	100	0	0
Azlocillin	100	0	0
Ticarcillin	100	0	0
Moxalactam	100	0	0
Cefuroxime	4	0	96
Cefotaxime	20	60	20
Cefoperzone	100	0	0
Ceftazidime	100	0	0
Ceftriaxone	64	12	24
Streptomycin	0	0	100
Kanamycin	4	0	96
Gentamicin	0	0	100
Amikacin	4	0	96
Tobramycin	0	0	100
Netilmicin	0	0	100
Trimethoprim/ Sulphamethoxazole	76	0	24
Chloramphenico'	20	60	20
Colistin	0	0	100
Penicillin (10)**	0	0	100
Tetracycline (25)	0	0	100
Erythromycin (15)	0	0	100
Polymyxin B (100)	68	0	32
Rifampicin (30)	4	8	88
Nalidixic acid (30)	72	8	20
Nitrofurantoin (200)	4	0	96

*Susceptibility to the first 20 antibiotics by MIC determinations.
**Susceptibility to the last 7 antibiotics by disc diffusion tests. The numbers in brackets denote disc concentration in units (Penicillin) and mg/L.

as per cent susceptibility or resistance with additional results for seven other drugs obtained by disc diffusion tests.

The 25 strains were fairly uniform in their susceptibility to B-lactams and resistance to aminoglycosides. All the strains were susceptible to ampicillin, piperacillin, azlocillin, ticarcillin, moxalactam, cefoperazone and ceftazidime and all except one to carbenicillin. Of these, azlocillin appeared to have the highest activity followed closely by moxalactam and piperacillin, all strains being inhibited by 2 mg/L or less of these three antibiotics. More resistances (20-30%) were encountered towards ceftriaxone, polymyxin B and nalidixic acid while most of the strains showed only moderate susceptibility to cefotaxime and chloramphenicol with median MICs of 12 and 11 mg/L respectively. Of the six aminoglycosides tested, tobramycin had the highest activity but as with the others, most of the strains were considered resistant with MICs over 8 mg/L. The majority of strains were also resistant to penicillin, cefuroxime, tetracycline, erythromycin, colistin, rifampicin and nitrofurantoin.

There appeared to be two sub-populations of *A. xylosoxidans* with regards to cotrimoxazole susceptibility. Nineteen (76%) of the strains were susceptible to 0.5 mg/L of trimethoprim and 9.5 mg/L of sulphamethoxazole tested as a 1:19 combination while the remainder six (24%) were resistant to 8 mg/L of trimethoprim and 152 mg/L of sulphamethoxazole. When the two drugs were tested separately, it was found that all the strains were resistant to trimethoprim while all except six were susceptible to sulphamethoxazole. Hence susceptibility to the combination largely depended on susceptibility to sulphamethoxazole.

DISCUSSION

Although *A. xylosoxidans* is still a rarely reported cause of hospital-acquired infections, it has the potential to become an important nosocomiant like *Pseudomonas aeruginosa* and *Serratia marcescens.* A major attribute will be its resistance to multiple antibiotics.

The antibiograms of this organism appear to be fairly consistent with most workers reporting resistance to aminogycosides (1, 3, 4) and sensitivity to carbenicillin (3, 5) and cotrimoxazole (4, 5, 6). However, while ampicillin-resistance is shown by most workers, Holmes et al (5) found 11 of their 14 strains to be sen-

sitive by disc diffusion tests and we found all our 25 strains to be sensitive by MIC determinations.

The seven strains isolated from community-acquired infections were not very different in their antibiotic susceptibilities from those isolated in the hospital. Like the hospital strains, they were also multi-resistant to 13 to 15 antimicrobials each. However, there were only three different antibiograms among the 18 hospital strains whereas four different antibiograms were obtained from the seven community strains. This probably reflects the circulation of a few endemic strains in our hospital environment compared to the presence of more heterogenous strains in the community.

A. xylosoxidans is unusual among gram-negative nosocomiants in its almost universal resistance to aminoglycosides. This suggests that conventional therapy with gentamicin or amikacin for severe gram-negative sepsis will not be effective against *A. xylosoxidans* infections. Fortunately, our strains were all sensitive to most B-lactam antibiotics. Hence combinations of B-lactams and aminoglycosides may still be suitable empirical treatment.

There appears to be good correlation between *in vitro* activity of the antimicrobials and *in vivo* response to treatment. Carbenicillin alone or in combination with kanamycin has been shown to be effective against *A. xylosoxidans* (4, 7) and cases of meningitis by this organism have been cured by chloramphenicol (3). In our own series, three cases of septicaemia died while on treatment respectively with cloxacillin and gentamicin, cefuroxime and gentamicin, and chloramphenicol. *In vitro* testing showed resistance to all four drugs. Five other cases of septicaemia recovered with cotrimoxazole, carbenicillin (2 patients), chloramphenicol and combination of ampicillin and gentamicin. In each case, *in vitro* testing showed susceptibility of the infecting strain to the drug used for therapy.

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