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Quality assessment of Microbe Base antimicrobial susceptibility data

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Abstract

***Aims*—To assess the quality of centres contributing antimicrobial susceptibility data to a centralised database.**

***Methods*—Twelve organisms were distributed to 31 regional microbiology laboratories contributing data to a centralised susceptibility database. Participants were asked to determine susceptibilities to certain antibiotics by their routine method and return the data to the Department of Microbiology, Royal Hallamshire Hospital, Sheffield, for analysis.**

***Results*—Results for the overwhelming majority of organism/antibiotic combinations were in agreement with expected results. Reasons for discrepancies included the non-bimodal distribution of susceptibilities, the use of different content discs, and, more importantly, minimum inhibitory concentrations falling close to breakpoint values.**

***Conclusions*—It is inevitable that any large multicentre database will contain a degree of inaccurate data. This study has highlighted several areas where discrepant results have occurred and has enabled Glaxo Laboratories to approach individual laboratories to address this problem. This study emphasises the value and consistency of Microbe Base as the largest database, of its kind, nationally.**

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Keywords: Antimicrobial susceptibility, database, data.

Epidemiological surveillance is an important function of microbiology laboratories. Microbe Base is a computer software suite,[1 2] developed in Sheffield, that allows microbiology laboratories to store and analyse antimicrobial susceptibility data using personal computers. At the Department of Microbiology, Royal Hallamshire Hospital, Sheffield, we have used these programs to perform computed surveillance for the past 14 years. We also recognised the programs' potential for collecting multicentre susceptibility data. Glaxo Laboratories distributed the program suite to 61 centres throughout the UK, producing the largest incidence and susceptibility survey to date.[3–5] They have now developed a number of individual formats so that anonymous patient data can be down-loaded from hospital computer systems onto floppy disks for transfer to the mainframe computer at Glaxo which houses the Microbe Base national database and currently holds in excess of one million records. Prior to this study, there had been no attempt to assess the quality of data stored in Microbe Base, other than individual laboratories' voluntary participation in external quality assurance schemes such as the National External Quality Assurance Scheme (NEQAS).[6] The aim of this study was to determine the suitability of centres contributing data towards the Microbe Base national database.

Methods

Twelve organisms were distributed to 31 laboratories routinely using Microbe Base for storage of antimicrobial susceptibility data. The organisms comprised three well characterised control strains: *Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662. These were said to be isolated from blood, urine and a wound, respectively. Eight clinical strains were said to be isolated from a variety of sites (*E coli* from blood; *S aureus* from a wound; *P aeruginosa* from blood; *Haemophilus influenzae* from blood and cerebrospinal fluid; *Klebsiella oxytoca* from a wound; *Enterobacter cloacae* from urine; and *Streptococcus pneumoniae* from cerebrospinal fluid). These were identified using standard laboratory procedures.[7 8] Minimum inhibitory concentrations (MICs) were determined using diagnostic sensitivity test (DST) agar (Unipath) supplemented, when necessary, with lysed horse blood (2%) and nicotinamide adenine dinucleotide (NAD, 20 mg/l), an inoculum of 10^5 organisms per spot, and incubation at 37°C for 18 hours. Methicillin MICs were determined at 30°C. Susceptibility was assigned by reference to the Working Party of the British Society for Antimicrobial Chemotherapy.[9] The three control organisms were chosen because most centres used these as controls for disc diffusion tests. The others were included to be representative of antibiotic resistance patterns found in common pathogens. The twelfth strain (organism 10) was found to be a mixed culture and was not included in the analysis. Participants were asked to determine susceptibilities to certain antibiotics only; these data were returned to the Department of Microbiology at the Royal Hallamshire Hospital for analysis.

Results

Of the 31 participating centres, 29 used disc diffusion as a routine susceptibility method; seven also used agar dilution and one also used

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Table 1 MIC values and percentage agreement for Staphylococcus aureus *from blood (strain 1) and wound (strain 6)*

Antimicrobial agents	*Strain number*	*MIC (mg/l)*	*Interpretation*	*No. of laboratories returning*	*% Agreement*
Gentamicin	1	0·25	Susceptible	31	100
	6	0·25	Susceptible	30	100
Cefuroxime	1	0·5	Susceptible	30	96·7
	6	32	Resistant	30	96·7
Penicillin	1	0·012	Susceptible	31	100
	6	8	Resistant	31	100
Erythromycin	1	0·25	Susceptible	30	100
	6	⩾16	Resistant	30	100
Methicillin	1	0·5	Susceptible	31	100
	6	⩾256	Resistant	31	100

Table 2 MIC values and percentage agreement for Escherichia coli *(strain 2) and* Enterobacter cloacae *(strain 8) from urine*

Antimicrobial agents	*Strain number*	*MIC (mg/l)*	*Interpretation*	*No. of laboratories returning*	*% Agreement*
Trimethoprim	2	0·03	Susceptible	31	100
	8	0·25	Susceptible	31	90·3
Ampicillin	2	2	Susceptible	30	100
	8	32	Resistant	30	86·7
Cephalexin	2	4	Susceptible	27	100
	8	32	Resistant	27	92·6
Ciprofloxacin	2	0·012	Susceptible	29	100
	8	0·03	Susceptible	29	100
Gentamicin	2	0·5	Susceptible	31	100
	8	0·25	Susceptible	31	96·8
Cefuroxime	2	2	Susceptible	31	100
	8	16	Susceptible	31	83·9
Ceftazidime	2	0·12	Susceptible	31	100
	8	0·5	Susceptible	31	100
Imipenem	2	0·06	Susceptible	28	100
	8	0·06	Susceptible	29	100
Amoxycillin/ clavulanate	2	4	Susceptible	29	100
	8	32	Resistant	28	78·6

Table 3 MIC values and percentage agreement for Escherichia coli *from blood (strain 9) and* Klebsiella oxytoca *(strain 11) from a wound*

Antimicrobial agents	*Strain number*	*MIC (mg/l)*	*Interpretation*	*No. of laboratories returning*	*% Agreement*
Trimethoprim	9	⩽0·12	Susceptible	31	100
	11	⩾32	Resistant	31	100
Ampicillin	9	⩾256	Resistant	30	100
	11	⩾256	Resistant	30	100
Cephalexin	9	16	Resistant	26	84·6
	11	16	Resistant	25	60·0
Ciprofloxacin	9	0·03	Susceptible	30	100
	11	2	Resistant	30	83·3
Gentamicin	9	0·5	Susceptible	31	100
	11	0·25	Susceptible	31	100
Cefuroxime	9	8	Resistant	31	58·1
	11	16	Resistant	30	86·7
Ceftazidime	9	8	Resistant	31	74·2
	11	0·5	Susceptible	31	87·1
Imipenem	9	0·06	Susceptible	30	100
	11	0·06	Susceptible	30	100
Amoxycillin/ clavulanate	9	16	Resistant	28	67·9
	11	16	Resistant	27	92·6

Table 4 MIC values and percentage agreement for Pseudomonas aeruginosa *from wound (strain 3) and blood (strain 7)*

Antimicrobial agents	*Strain number*	*MIC (mg/l)*	*Interpretation*	*No. of laboratories returning*	*% Agreement*
Ciprofloxacin	3	0·06	Susceptible	30	100
	7	0·12	Susceptible	30	86·7
Gentamicin	3	0·5	Susceptible	31	100
	7	4	Resistant	31	29·0
Ceftazidime	3	1	Susceptible	31	96·8
	7	1	Susceptible	31	96·8
Imipenem	3	0·12	Susceptible	30	100
	7	0·06	Susceptible	30	100

VITEK (BioMerieux, France). One laboratory used agar dilution alone and one used ATB (BioMerieux) alone. Of the 29 centres using disc diffusion, 25 used a modified Stokes' technique, two used the comparative method[10] and two used the Kirby–Bauer method.[11]

Consensus agreement between participants and the central laboratory is shown in tables 1–6. Organisms 1 and 6 were strains of *S aureus* said to be isolated from blood and wound sites, respectively. In reality, organism 1 was NCTC 6571 (the Oxford staphylococcus) and organism 6 was a methicillin resistant staphylococcus (EMRSA-15). Results from all centres were in accordance with expected results for both organisms tested against gentamicin, penicillin, erythromycin, and methicillin. However, one centre reported false resistance of strain 1 to cefuroxime. This was surprising as this centre used the comparative technique with 30 μg discs and NCTC 6571 as control; it was, however, the only laboratory not to use a suspending fluid for the inoculum. One centre reported strain 6 as susceptible to cefuroxime (but resistant to methicillin): this centre used methodology similar to that used by others (Stokes' technique with 30 μg discs).

Organism 2 was a strain of *E coli* said to be isolated from urine. In reality, this was NCTC 10418 and all centres found the organism to be susceptible to trimethoprim, ampicillin, cephalexin, ciprofloxacin, gentamicin, cefuroxime, ceftazidime, imipenem, and amoxycillin/clavulanate. All centres found organism 8 (*E cloacae* from urine) to be susceptible to ciprofloxacin, ceftazidime and imipenem. Three centres, using typical disc strengths of 1·25 or 2·5 μg, reported that this organism was resistant to trimethoprim despite a MIC of 0·25 mg/l. Two centres reported the strain as susceptible and two as intermediate resistance to ampicillin (MIC 32 mg/l). Two centres also found this strain susceptible to cephalexin despite a MIC of 32 mg/l. One centre reported false resistance to gentamicin (MIC 0·25 mg/l). Five centres reported resistance to cefuroxime despite a MIC of 16 mg/l. Three centres reported intermediate resistance and three reported susceptibility to amoxycillin/clavulanate; the majority reported resistance, confirming the MIC of 32 mg/l.

Organisms 9 and 11 were strains of Enterobacteriaceae said to be isolated from blood (*E coli*) and a wound (*Kl oxytoca*). There was excellent agreement with expected results when both strains were tested against trimethoprim, ampicillin, gentamicin, and imipenem. Four centres reported that strain 9 was susceptible to cephalexin; the central laboratory recorded a MIC of 16 mg/l, resistant by British Society for Antimicrobial Chemotherapy criteria (BSAC, 1991). Ten centres reported that strain 11 was susceptible to cephalexin, five reported intermediate susceptibility and 10 reported resistance: the MIC was 16 mg/l. Five centres reported that strain 11 was susceptible to ciprofloxacin (MIC 2 mg/l), three of them using high strength (5 μg) discs, and four centres reported susceptibility to cefuroxime (MIC 16 mg/l). All centres performing disc tests with ceftazidime used 30 μg content discs. Organism 11 was fully susceptible to ceftazidime (MIC 0·5 mg/l) but three centres reported intermediate resistance and one reported resistance. Eight centres found that organism 9 was susceptible to ceftazidime (MIC 8 mg/l). Two centres reported that strain 11 was susceptible to amoxycillin/clavulanate (MIC

Table 5 MIC values and percentage agreement for Haemophilus influenzae *from blood (strain 4) and cerebrospinal fluid (strain 12)*

Antimicrobial agents	*Strain number*	*MIC (mg/l)*	*Interpretation*	*No. of laboratories returning*	*% Agreement*
Trimethoprim	4	≤0·12	Susceptible	30	93·3
	12	0·25	Suscepitble	31	100
Ampicillin	4	≥256	Resistant	29	100
	12	0·25	Susceptible	30	96·7
Cefuroxime	4	0·5	Susceptible	30	100
	12	0·5	Susceptible	31	93·5
Erythromycin	4	8	Resistant	28	82·1
	12	4	Resistant	28	64·3
Chloramphenicol	4	0·5	Susceptible	27	100
	12	8	Resistant	28	89·3
Amoxycillin/ clavulanate	4	1	Susceptible	28	67·9
	12	0·5	Susceptible	29	100
β-lactamase	4	Positive		30	100
	12	Negative		31	100

Table 6 MIC values and percentage agreement for Streptococcus pneumoniae *from cerebrospinal fluid (strain 5)*

Antimicrobial agents	*Strain number*	*MIC (mg/l)*	*Interpretation*	*No. of laboratories returning*	*% Agreement*
Ciprofloxacin	5	2	Resistant	25	72·0
Cefuroxime	5	2	Susceptible	26	76·9
Penicillin	5	0·25	Resistant	27	100
Erythromycin	5	0·25	Susceptible	26	100

16 mg/l). The disparity among amoxycillin/clavulanate results for strain 9 may also be explained by a MIC of 16 mg/l, a value close to the breakpoint of 8 mg/l.

Organisms 3 and 7 were strains of *P aeruginosa* said to be isolated from a wound and blood, respectively. Organism 3 was, in fact, NCTC 10662 and fully susceptible to gentamicin (MIC 0·5 mg/l), ceftazidime and imipenem. Organism 7 was also susceptible to ciprofloxacin, ceftazidime and imipenem but demonstrated a raised MIC for gentamicin (4 mg/l). By BSAC criteria, strain 7 was resistant to gentamicin; this was confirmed by distribution of this particular strain to 23 laboratories performing susceptibility tests using the agar dilution technique (data not shown). In the present study, in which disc tests were predominantly used, only four centres reported intermediate resistance; a further five reported resistance. The remaining 22 centres reported the strain as fully susceptible. Two centres reported intermediate resistance and two reported resistance to ciprofloxacin with strain 7. The same centre reported false ceftazidime resistance in both strains. All centres reported both strains as susceptible to imipenem and strain 3 as susceptible to gentamicin.

Organisms 4 and 12 were strains of *H influenzae* said to be isolated from blood and cerebrospinal fluid, respectively. Organism 4 produced β-lactamase whereas organism 12 did not; all centres examining these strains determined this correctly. Correlation between ampicillin susceptibility and enzyme production was excellent. One centre reported false ampicillin resistance in strain 12. All centres found that the β-lactamase negative strain was susceptible to amoxycillin/clavulanate, although nine reported the β-lactamase producer as resistant. According to the central laboratory, this strain was susceptible by both National Committee for Clinical Laboratory Standards[12] and BSAC criteria although the MIC corresponded to the BSAC breakpoint (1 mg/l). Two centres reported false resistance to trimethoprim in strain 4 and two reported false resistance to cefuroxime in strain 12. Strain 4 was correctly designated as chloramphenicol susceptible by all centres; however, three reported false susceptibility in strain 12. One of these centres used high content (30 μg) discs compared with the more usual content of 10 μg. There was no clear consensus on erythromycin susceptibility with five centres reporting susceptibility in strain 4, and 10 centres in strain 12.

Organism 5 was a strain of *S pneumoniae* said to be isolated from cerebrospinal fluid. All centres correctly reported the strain as resistant to penicillin and susceptible to erythromycin. However, seven (of 25) centres reported the strain as susceptible and two as intermediate resistance to ciprofloxacin. Six (of 26) reported the strain as cefuroxime resistant (MIC 2 mg/l).

Discussion

Antimicrobial susceptibility testing is used to assist in the choice of antimicrobial therapy; spurious results may, therefore, directly affect patient management. For this reason, most laboratories use documented methodologies—for example, Stokes' method, the comparative method, NCCLS, BSAC, or British Society for Microbial Technology (BSMT)[13] guidelines. Although we did not set out to determine directly the reason for discrepant results between centres, some became apparent as part of the study. Most centres used disc diffusion tests controlled by the standard organisms, NCTC 6571, NCTC 10418 and NCTC 10662. Failure to obtain correct (susceptible) results with these strains could, therefore, invalidate all comparative tests. This exercise revealed that one centre was interpreting *S aureus* NCTC 6571 as cefuroxime resistant and *P aeruginosa* NCTC 10662 as ceftazidime resistant. This was mirrored in results for four quality control strains (strains 7, 8, 11, and 12).

There was no clear consensus on erythromycin susceptibility in *H influenzae*; this probably reflects the fact that these susceptibilities are not bimodally distributed. Other discrepancies were caused by laboratories using different disc contents—for example, ciprofloxacin with *S pneumoniae* strain 5. All centres reporting susceptibility used 5 μg content discs; of the two centres reporting intermediate resistance, one used 5 μg discs and the other breakpoints. However, of 16 centres reporting resistance, 11 used 1 μg discs and five 5 μg discs. The majority of discrepant results occurred with antibiotic MICs falling on, or very near to, prescribed breakpoint values. This phenomenon has also been noted by others (Jenks P, personal communication), who suggested that individual centres amend their methods to use laboratory specific guidelines for interpretation of zone sizes. Strain 7 demonstrated a MIC of 4 mg/l for gentamicin. In this study, four centres reported intermediate resistance and a further five full resistance. Of

these, three used the agar dilution method of susceptibility testing. However, 22 centres using disc diffusion methods reported this strain as susceptible to gentamicin. The fact that strains of *P aeruginosa* clinically resistant to gentamicin may be classed as susceptible by this method but as intermediate or resistant by agar dilution methods has previously been noted.[13]

Had the strains in this study been from clinical samples, some results may have profoundly affected patient management. For example, strain 12 was a chloramphenicol resistant strain of *H influenzae*, said to be isolated from cerebrospinal fluid. Three centres reported this organism as spuriously susceptible.

It is inevitable that a large multicentre database such as Microbe Base will contain a degree of inaccurate data. Omitting strain 7 (as already discussed), we estimate the error in this study to be 6% of organism/antibiotic combinations. This is not to say that the error in Microbe Base data is also 6%. Participating centres knew that these organisms were control strains, and their analysis represented a relatively narrow range of organism/antibiotic combinations.

This exercise has enabled Glaxo Laboratories to approach individual laboratories to address this problem, and emphasises the value and consistency of Microbe Base as the largest database, of its kind, nationally.

Microbe Base is a Glaxo Laboratories trade mark.

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