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Occurrence and Expression of Imipemide (*N*-Formimidoyl Thienamycin) Resistance in Clinical Isolates of Coagulase-Negative Staphylococci

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More than 500 clinical isolates were screened for resistance to a number of antibiotics, including imipemide (*N*-formimidoyl thienamycin [MK0787]). Of the 25 coagulase-negative staphylococcal isolates present in the screening sample, almost one-third showed one of two patterns of imipemide resistance. One pattern apparently involves constitutive expression of drug resistance, whereas the other pattern seems to result from an inducible resistance having an apparent induction threshold higher than the minimal inhibitory concentration of imipemide. The mechanism(s) responsible for this imipemide resistance is unclear, but may be distinct from the more common staphylococcal mechanisms of resistance to β-lactam antibiotics. Only two of the patients from whom imipemide-resistant staphylococci were cultured had actually been treated with the antibiotic.

We report the observation and primary characterization of clinically isolated bacteria resistant to the antimicrobial agent imipemide (*N*-formimidoyl thienamycin [MK0787]), the stabilized derivative of a β-lactam molecule produced by *Streptomyces cattleya*. The unstable, original antibiotic thienamycin was isolated in 1976, and as yet thienamycin and imipemide have had only limited clinical use (14; K. J. Wildonger, W. J. Leanea, T. W. Miller, and B. G. Christensen, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 232, 1979). Imipemide is resistant to β-lactamases produced by a wide variety of bacterial strains and has been shown in vitro to inhibit the activity of several purified β-lactamases (12, 40). Although many bacterial species have been screened for resistance to imipemide. only in *Pseudomonas maltophilia* has such resistance been found routinely (13, 15, 23, 24, 38, 41).

The imipemide-resistant organisms reported here were predominantly coagulase-negative staphylococci, although the majority of the coagulase-negative staphylococci screened were susceptible to the drug. In some cases the resistance to imipemide appeared to be inducible, even in strains apparently constitutive for ampicillin and oxacillin resistance. In the absence of widespread use of imipemide, it is surprising to find a significant frequency of resistance to this antibiotic in a species that is usually susceptible to it. In response to these results, we have begun to investigate the nature of this imipemide resistance.

(A preliminary account of these findings was presented previously [R. Raeder, R. M. Blumenthal, C. D. Takemoto, and E. H. Freimer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I107, p. 157].)

MATERIALS AND METHODS

Bacterial strains. All strains were isolated from patients at the Medical College of Ohio Hospital between March and May 1982. Single-colony staphylococcal isolates were demonstrated to be gram-positive, catalase-positive cocci and were tested for coagulase activity. Coagulase-negative staphylococcal isolates were characterized further. Tentative species designations were determined through measuring the susceptibility of the isolates to novobiocin and lysostaphin (17).

The novobiocin and lysostaphin were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Antibiotics. Antibiotics used in Kirby-Bauer disk susceptibility assays included carbenicillin (100 μg), cephalothin (30 μg), erythromycin (15 μg), gentamicin (10 μg), penicillin G (10 μg), and oxacillin (1 μg) from Pfizer, Inc. (New York, N.Y.), as well as vancomycin (30 μg; General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.), ampicillin (10 μg; General Diagnostics), imipemide (10 μg; Merck Sharp & Dohme, Rahway, N.J.), moxalactam (30 μg; Eli Lilly & Co., Indianapolis, Ind.), ticarcillin (75 μg; BBL Microbiology Systems, Cockeysville, Md.), and ceftizoxime (30 μg; BBL).

Imipemide (powder) was provided by Merck Sharp & Dohme, and stocks of 100 μg/ml or 5 mg/ml were

prepared as recommended in 0.5 M morpholinopropanesulfonate (pH 6.8)–25% ethylene glycol and stored at −80°C.

Disk susceptibility assays. The disk susceptibility assays were performed as described by Bauer et al. (2), using Mueller-Hinton agar (Difco Laboratories, Detroit, Mich., or prepoured 15-cm plates from GIBCO Laboratories, Madison, Wis.). Resistance to mercury (as $HgCl_2$) or cadmium [as $Cd(NO_3)_2$] was determined by placing 20 μl of a 1 mM solution onto a sterile disk (Difco).

MIC determination. The minimal inhibitory concentrations (MICs) were determined by a microtiter broth dilution technique in which microtiter trays containing twofold dilutions of the antibiotic in Mueller-Hinton medium were inoculated with 50 μl of culture. The inoculum was an overnight culture diluted to 2×10^5 viable organisms per ml in Mueller-Hinton broth. The MIC was the lowest concentration of the antibiotic preventing visible growth of the organisms within 24 h at 37°C.

Growth experiments. All growth experiments were performed in Mueller-Hinton broth (Difco), with growth monitored as the absorbance at 540 nm in a Coleman 54B spectrophotometer (Coleman Inst., Maywood, Ill.). The inoculum was always a culture in exponential growth. Cultures (100 ml in 500-ml sidearm flasks) were shaken at 37°C and were shifted through the addition, at time 0, of various amounts of imipemide. Since undiluted readings were made, there was some nonlinearity in absorbance readings at 540 nm of about 0.2 or higher.

RESULTS

Imipemide resistance in clinical isolates. In extending an earlier study (24), we began a routine screening of bacterial strains for the detection of resistance to the newer β-lactam antibiotics, including imipemide. The strains examined were all clinical isolates and included a broad range of bacterial species. The vast majority of these strains proved to be susceptible to imipemide.

Mueller-Hinton agar was used because it is the standard medium for Kirby-Bauer tests (2). This medium contains a significant amount of cysteine, and imipemide is somewhat sensitive to such β-aminothiols. Nevertheless, in control experiments L-cysteine was added to Mueller-Hinton plates to final concentrations 0.025 to 0.20 mM above normal, and no changes were observed in the zones of inhibition around imipemide disks for either of the two strains tested (data not shown).

Susceptibility was defined either by a zone of inhibition with a diameter greater than 16 mm in standard Kirby-Bauer tests with a disk containing 10 μg of imipemide, or by an MIC lower than 20 μg/ml. (These definitions of imipemide susceptibility are those recommended by the manufacturer.) Of the 544 isolates screened, only 29 appeared to be resistant to imipemide; 20 of these were *P. maltophilia*, 1 was *Streptococcus faecalis*, and 8 were coagulase-negative staphylococci.

Imipemide resistance in *Pseudomonas* species has been reported previously (13, 15, 23, 24, 38, 41). However, the proportion of coagulase-negative staphylococci found to show some pattern of imipemide resistance (8 of 25, or 32%) was unexpected and prompted further studies.

Comparison of imipemide-resistant and -susceptible staphylococci. Antibiotic resistance profiles and preliminary species identification of the 25 isolates of coagulase-negative staphylococci were determined. No clear correlation could be made between resistance or susceptibility to imipemide and any other trait. All of the isolates appeared to be either *Staphylococcus epidermidis* (12 of 25) or *Staphylococcus simulans*, and both species were represented in the imipemide-resistant and -susceptible groups. All of the imipemide-resistant strains were also resistant to penicillin, ampicillin, oxacillin, carbenicillin, moxalactam, ticarcillin, ceftizoxime, and erythromycin, but not all strains resistant to these antibiotics were also resistant to imipemide. Nor was any correlation evident between imipemide resistance and resistance to cephalothin, gentamicin, tetracycline, vancomycin, mercury, or cadmium.

The frequencies of resistance to various antibiotics among those 25 isolates were not grossly different from the frequencies measured, for a larger group of coagulase-negative staphylococci, by the clinical microbiology laboratory at the Medical College of Ohio Hospital. Those frequencies that did differ significantly between the two collections of staphylococci were not due to differences between imipemide-resistant and -susceptible strains.

Two patterns of imipemide resistance. There were two distinct classes of imipemide-resistant staphylococci, distinguishable by their pattern of growth around an imipemide disk. The members of one class (3 of 25 isolates) showed a zone of inhibition of 6 to 16 mm and were termed Imi^r. The second class (5 of 25 isolates) grew immediately around the imipemide disk, but had a zone of inhibition farther out (see Fig. 1). These were termed Imi^{dz} (double zone). The variation in response of three Imi^{dz} isolates is shown in Fig. 1. The observed differences in inner and outer zone diameters and in the amount of background growth are consistent but highly dependent on inoculum size and medium composition, and may result from small differences in the growth rates of the isolates. Typically, a disk containing 10 μg of imipemide will cause Imi^{dz} strains to grow within 15 to 20 mm of the disk's edge, surrounded by a 10- to 20-mm-wide ring of no growth.

Since new plates inoculated with bacteria

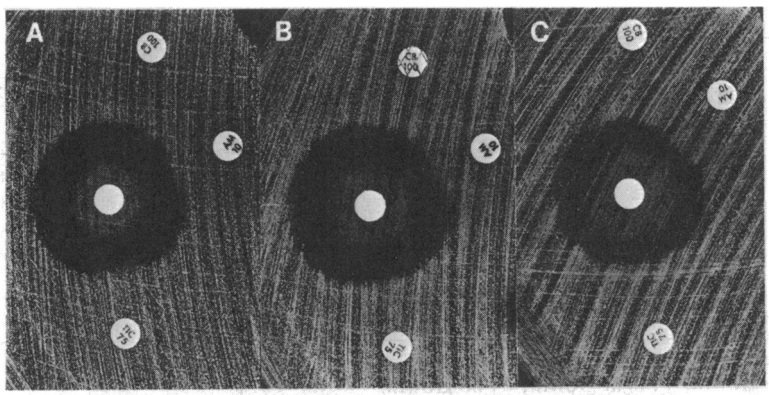

FIG. 1. Three examples of the Imi^{dz} phenotype. These three coagulase-negative staphylococcal isolates show some of the consistent differences seen among various Imi^{dz} strains. The differences include variations in the inner and outer diameters of the zone of inhibition and in the amount of growth that occurs within that ring. The unmarked disk contained 10 μg of imipemide.

from either the inner or outer zone of growth yielded the same double zone around imipemide disks, this Imi^{dz} phenomenon was not due to a genetic selection.

Double-zone resistance to imipemide. The double-zone pattern of resistance was investigated in a number of other ways. First, the response of Imi^{dz} isolates to a range of imipemide concentrations was determined by using the Kirby-Bauer procedure (Fig. 2). The Imi^{dz} isolates grew with a simple zone of inhibition around disks with low amounts of imipemide. As the amount of drug on the disk was increased, the zone expanded and growth around the disk began to appear.

Second, standard determinations of the imipemide MIC for some of these isolates showed the same pattern: growth at both ends of the concentration range with little or no growth detected at intermediate drug concentrations (Table 1). For three of the Imi^{dz} isolates (strains 9679, 8730,

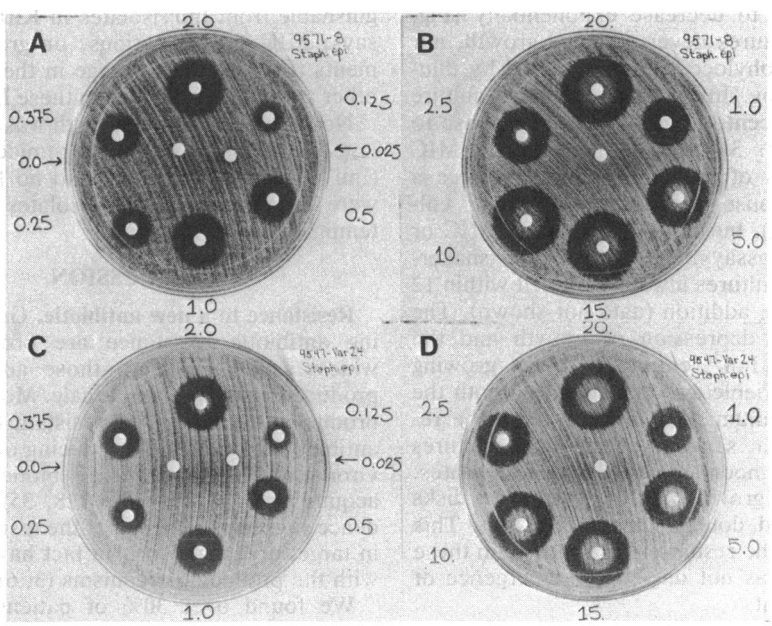

FIG. 2. Concentration dependence of the Imi^{dz} phenotype. The effects of a range of imipemide concentrations on the growth of two Imi^{dz} isolates, *S. epidermidis* 9571-8 (A, B) and *S. simulans* 9547-24 (C, D), are shown. The low-concentration series (A, C) had disks with 0, 0.025, 0.125, 0.250, 0.375, 0.500, 1.00, and 2.00 μg of imipemide. The higher-concentration series (B, D) had disks with 0 (center), 1.00, 2.50, 5.00, 10.0, 15.0, and 20.0 μg of imipemide. The disks with no drug contained only imipemide stabilizing buffer.

TABLE 1. Imipemide MIC assays

S. epidermidis isolate[a]	Phenotype	Growth[b] at imipemide concn (μg/ml):												
		100	50	40	30	10.0	8.0	6.0	4.0	2.0	1.0	0.9	0.8	0.7
7205	Imi^r	−	−	−	−	−	−	−	−	−	−	−	−	+
7949	Imi^r	−	−	−	++	++	++	++	++	++	++	++	++	++
6346	Imi^{dz}	−	++	++	++	++	++	++	++	++	++	++	++	++
9679	Imi^{dz}	−	++	++	++	++	++	++	++	++	++	++	++	+
9571	Imi^{dz}	−	++	++	++	++	++	++	++	++	−	−	−	−
8730	Imi^{dz}	−	++	++	++	++	++	++	++	++	+	+	+	+
9311	Imi^s	−	−	−	−	−	−	−	−	−	−	−	−	−
8324	Imi^s	−	−	−	−	−	−	−	−	−	−	−	−	−
8163	Imi^s	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] Isolates 7205, 9311, and 8324 are *S. simulans*.
[b] ++, Heavy growth; +, light growth; −, no growth.
[c] No viable bacteria could be recovered by streaking onto Trypticase soy agar (see text).

and 9571), 1-μl samples of the MIC cultures were streaked onto Trypticase soy agar plates containing no antibiotic. Strains 9679 and 9571 both had cultures at intermediate imipemide concentrations from which no viable bacteria could be recovered (Table 1). Some of the Imi^{dz} isolates, however, grew like Imi^r isolates in these determinations.

Third, growth-shift experiments were performed in which various amounts of imipemide were added to exponentially growing cultures of Imi^s or Imi^{dz} isolates (Fig. 3 and 4). As expected, the growth of Imi^s cultures rapidly slowed in response to as little as 0.5 μg of imipemide per ml, and after about 1 h the turbidity of the culture began to decrease exponentially (Fig. 3A). Such cultures never resumed growth, nor could live staphylococci be recovered by dilution and plating. In contrast, the Imi^{dz} culture showed a concentration-dependent response to the drug (Fig. 3B and 4A). Some sub-MIC concentrations of imipemide could produce a pronounced pause in the growth of Imi^{dz} cultures (Fig. 3B), but unlike the case of MIC or Kirby-Bauer assays, growth of the shaker-grown liquid cultures always resumed within 12 h of imipemide addition (data not shown). The extent of the depression of growth and the ability of the Imi^{dz} culture to begin growing again were variable and dependent on both the strain and medium composition. After the restart of growth, samples of the Imi^{dz} cultures were used to inoculate Mueller-Hinton plates. The resulting growth around imipemide disks again produced double zones (Fig. 5A). This indicates that the resumption of growth in these experiments was not due to the emergence of resistant mutants.

"Standard" resistance to imipemide. In contrast to the Imi^{dz} isolates, the expression of imipemide resistance by Imi^r isolates was independent of the drug concentration. In Kirby-Bauer assays, there was no zone of inhibition (10-μg imipemide disks). In MIC determinations, Imi^r isolates grew uniformly at all concentrations of imipemide below the MIC (Table 1). In growth experiments, the growth of Imi^r cultures was not significantly affected by the addition of 0.1 to 5.0 μg of imipemide per ml.

Derivation of Imi^r variants from Imi^{dz} isolates. One difference among the Imi^{dz} isolates was the amount of background growth in the zone of inhibition ringing an imipemide disk (Fig. 1). Colonies taken from this zone and used to inoculate new plates showed considerable growth in the zone of imipemide inhibition (Fig. 5B). Repeating this process serially led to the isolation of strains that were essentially indistinguishable from Imi^r isolates in Kirby-Bauer assays, MIC determinations, or growth experiments (Fig. 4B). No change in the response to other antibiotics was seen in these Imi^r variants.

No such background growth was seen around imipemide disks when imipemide-susceptible (Imi^s) isolates were used, and no Imi^r variants were obtained from Imi^s isolates through attempts at serial passage.

DISCUSSION

Resistance to a new antibiotic. Genes specifying antibiotic resistance are probably fairly widespread well before those antibiotics are produced on an industrial scale. Most antibiotic-producing organisms are resistant to their own antibiotics (7, 42). Nonproducing organisms environmentally exposed to antibiotics have often acquired resistance genes (28, 35). Other evidence suggests that some of the resistance genes in target organisms may in fact have originated with the producing organisms (3, 6).

We found over 30% of patient isolates of coagulase-negative staphylococci to be resistant to imipemide, yet only two of the patients carrying an imipemide-resistant strain were actually being treated with this drug. Previous studies of coagulase-negative staphylococci report imipe-

TABLE 1—*Continued*

0.6	0.5	0.4	0.3	0.2	0.10	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0.00
+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++
++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
+[c]	+[c]	++	++	+	++	++	++	++	++	++	++	++	++	++	++
−	−[c]	−[c]	−[c]	−[c]	++	++	++	++	++	++	++	++	++	++	++
+	+	+	+	+	+	++	++	++	++	++	++	++	++	++	++
−	−	−	−	−	+	−	+	++	++	++	++	++	++	++	++
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++
−	−	−	−	+	+	−	−	−	−	−	−	−	++	++	++

mide MIC values for 90% of strains ranging from 0.125 to 2.0 μg/ml (13, 15, 23, 38, 41). One notable exception is the study of Tally et al. (38), who found 3 of 20 isolates (15%) with an MIC as high as 32 μg of imipemide per ml. The majority of coagulase-negative staphylococci are susceptible to imipemide (our results; 13, 15, 23, 38, 41). Thus, the resistant subpopulation probably possesses genetic information, lacking in the majority of coagulase-negative staphylococci, that confers the drug resistance. This imipemide resistance could be an example of the limited spread of antibiotic resistance genes even before the relevant antibiotics have received widespread use.

Nature of imipemide resistance. Thienamycin-resistant mutants of *Escherichia coli* have been selected and found to have altered penicillin-binding proteins (36). In contrast, the mechanism of imipemide resistance has not been determined for either *P. maltophilia* or coagulase-negative staphylococci. In organisms that do not produce β-lactam antibiotics, the most common mechanism for resistance to these antibiotics is the production of a β-lactamase (25). Imipemide is an effective inhibitor of a wide variety of β-lactamases (40). Type IV plasmid-mediated penicillinase from *Pseudomonas aeruginosa* was not inhibited by imipemide (34, 40). Nevertheless, *P. aeruginosa* has been found to be almost uniformly susceptible to imipemide (12, 13, 21, 23, 24, 38, 41) (the resistant species is *P. maltophilia*). Furthermore, the resistance or susceptibility to a β-lactam antibiotic has not always

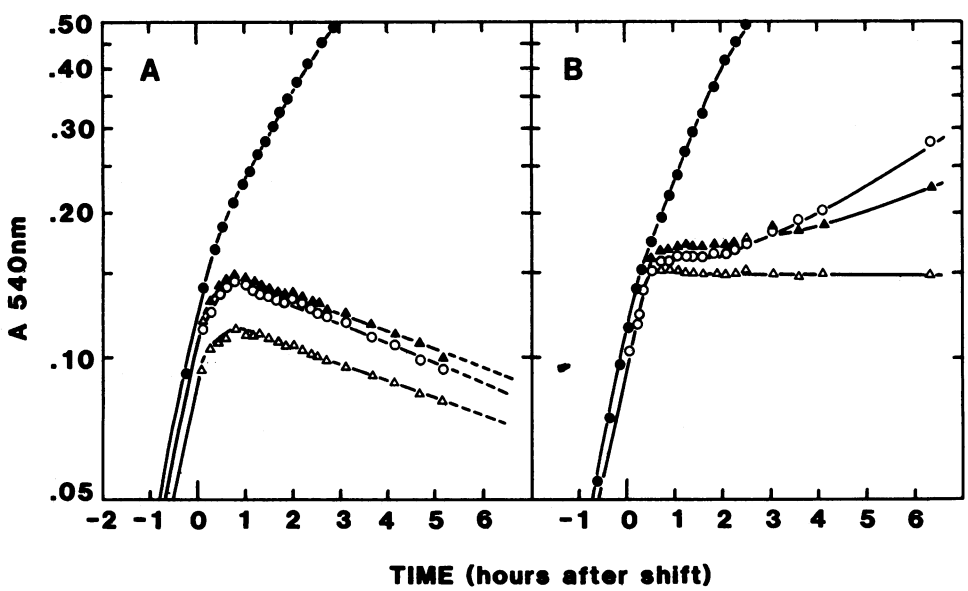

FIG. 3. Growth responses to imipemide of Imi[s] and Imi[dz] isolates. At time 0, imipemide was added to the culture to a final concentration of (●) 0, (○) 0.5, (▲) 1.0, or (△) 5.0 μg/ml. (A) *S. simulans* 94-7856 (Imi[s]). None of the inhibited cultures resumed growth within 12 h. (B) *S. epidermidis* 9571 (Imi[dz]). The culture with 5.0 μg/ml resumed growth within 12 h. A 540nm, Absorbance at 540 nm.

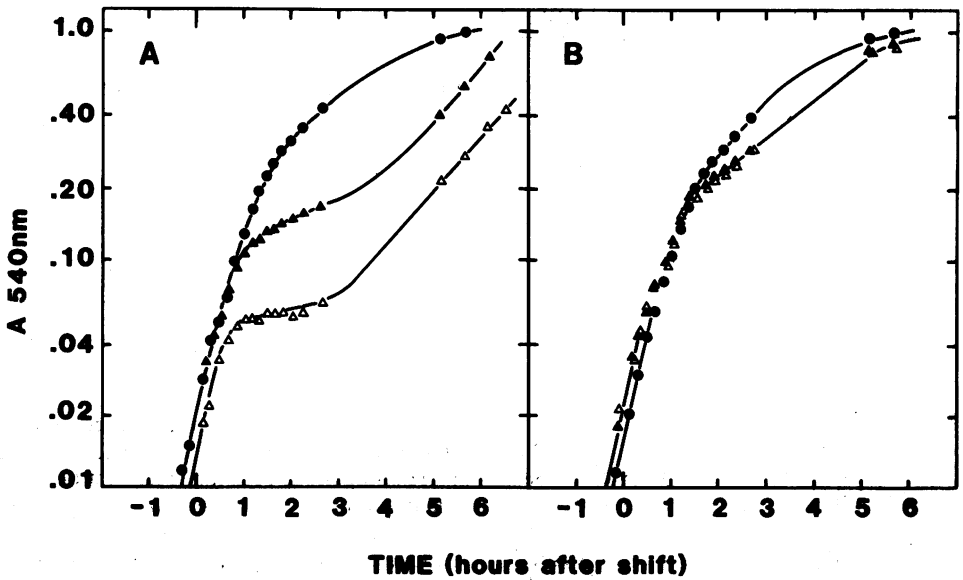

FIG. 4. Growth response of Imi^{dz} parent and "mid-zone" derivative. A variant of strain 9571, such as may be seen growing in the zone of inhibition in Fig. 1 or 5B, was compared with its parental strain in growth experiments. At time 0, imipemide was added to final concentrations of (●) 0, (▲) 0.3, or (△) 0.6 μg/ml. (A) Parental isolate 9571 (Imi^{dz}). (B) Derivative 9571-M. A 540nm, Absorbance at 540 nm.

been found to be a simple function of the presence or absence of a β-lactamase active against that drug (25, 32, 39).

In staphylococci, resistance to β-lactam antibiotics is generally due to four β-lactamases, usually specified by plasmid-borne genes (25). The A, B, and C β-lactamases are inducible, whereas the D-type β-lactamase seems to be expressed constitutively (29). Thus, the D-type β-lactamase is probably not involved in the resistance of Imi^{dz} isolates to imipemide. No isolates of *Staphylococcus aureus* have been reported to be resistant to imipemide (13, 15, 23, 38, 41). Thus, the common A-, B-, and C-type β-lactamases are probably not solely responsible for the imipemide resistance either. The other major form of resistance to β-lactam agents found in the staphylococci is the intrinsic resistance to methicillin (18, 19, 31). Methicillin resistance in *S. aureus* is not due to a β-lactamase, although the enzyme can play a peripheral role (30, 37). This methicillin resistance has been found to be constitutive (5). Whereas all of the coagulase-negative staphylococci found to be imipemide resistant were also resistant to methicillin (oxacillin), several of the imipemide-susceptible strains were also resistant to oxacillin. Furthermore, other studies report methicillin-resistant staphylococci to be susceptible to imipemide (21, 23). Thus it is possible that the imipemide resistance in these coagulase-negative staphylococci is not directly due to either the known staphylococcal β-lactamases or to the methicillin resistance system.

Expression of imipemide resistance. Two patterns of imipemide resistance were found in the coagulase-negative staphylococci. One pattern appears to be a constitutive expression. In growth experiments there were no detectable pauses in the growth of Imi^{r} isolates after the addition of imipemide. This pattern could, alternatively, be due to the rapid induction of a fast-acting resistance mechanism, but this seems less likely.

The second pattern of imipemide resistance seems to be due to an inducible system. The growth-shift experiments indicate that a pause in growth occurs after a short period of exposure to imipemide, and then eventually growth resumes (Fig. 3 and 4). This phenomenon is highly dependent on the concentration of the drug. The pattern of growth around an imipemide disk suggests that the concentration of the drug sufficient to induce the resistance mechanism is higher than the MIC of that drug. Interestingly, ampicillin and ticarcillin appear to affect the expression of imipemide resistance (Fig. 1A; unpublished data). This effect may be due to antagonism between the antibiotics, but the ability of one antibiotic to induce resistance to other functionally related antibiotics is a well-documented alternative explanation of this phenomenon (20). The ability to isolate Imi^{r} variants from Imi^{dz} strains is itself interesting. Such variants

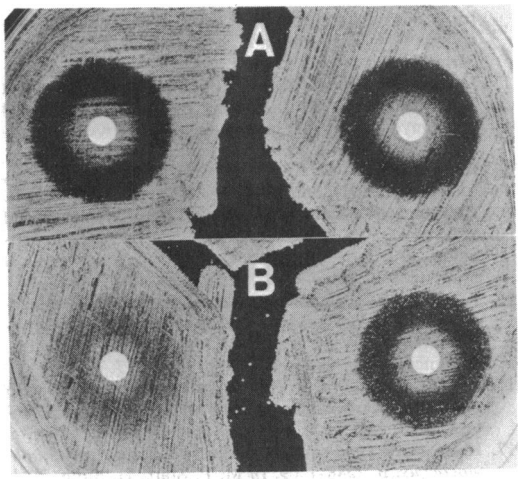

FIG. 5. Genetic stability of Imi^{dz} phenotype. (A) Response to imipemide of cultures taken before and after use in a growth experiment. Two portions of a Mueller-Hinton plate were spread with cultures of *S. epidermidis* 9571. On the left is the culture used as an inoculum for the experiment shown in Fig. 4A. On the right is the culture, from that experiment, which resumed growth in the presence of 0.6 μg of imipemide per ml. In both cases, the disk contained 10 μg of imipemide. (B) Behavior of mid-zone variants. *S. simulans* 9547 (Imi^{dz}) tends to have a relatively high frequency of variants growing within the zone of inhibition (right). If one of these variants was used as an inoculum, the zone of inhibition was considerably less pronounced but was still evident (left). The disks contained 10 μg of imipemide. This phenomenon has been seen with several other Imi^{dz} isolates as well.

may be defective for a repressor controlling the expression of imipemide resistance, for example.

There is one notable inconsistency in the data from Imi^{dz} strains. The distinct absence of growth shown by such strains at intermediate imipemide concentrations, and seen in MIC and Kirby-Bauer assays, was no more than a lag period in shaken liquid cultures. Mueller-Hinton-based media were used in all of these experiments. The imipemide itself appears to be equally stable in both types of cultures, so this may represent a physiological difference in the staphylococci due, perhaps, to the difference in aeration.

In preliminary experiments (not shown), imipemide-susceptible strains were used in bioassays of imipemide levels. No evidence of inactivation of the drug was found with Imi^{r} or Imi^{dz} strains, either by growth in the presence of the antibiotic or by incubation of imipemide with extracts of drug-exposed cultures. In contrast, inactivation of imipemide by extracts of *P. maltophilia* was easily detected (our results; 33). It is unlikely that the staphylococcal resistance to imipemide reported here is due to the activity of a β-lactamase. The possibility that a β-lactamase is causing resistance by binding and sequestering the drug, however, cannot yet be ruled out (39).

The Imi^{dz} phenotype and the Eagle effect. The ability of a homogeneous bacterial population to survive high and low, but not intermediate, concentrations of penicillin has been known for some time (8, 9). However, this "Eagle effect" is probably not directly related to the Imi^{dz} phenotype reported here. First, the Eagle effect is seen at very high levels of penicillin (e.g., 1,200 μg/ml), whereas 2 to 50 μg of imipemide per ml (a pharmacologically attainable level) is sufficient to elicit the expression of resistance in Imi^{dz} isolates. Second, neither Imi^{s} isolates nor Imi^{s} variants of Imi^{dz} isolates exhibit resistance around disks containing 10 to 25 μg of imipemide, so the Imi^{dz} phenomenon is probably due to an active resistance mechanism. And third, the Imi^{dz} strain 9571, when exposed to 2 μg of imipemide per ml, does not stop growing. The Eagle effect results in cells that remain viable but stop growing until the penicillin is removed. In summary, the imipemide resistance described here seems most likely to be due to inducible (Imi^{dz}) or constitutive (Imi^{r}) expression of active resistance mechanism(s), although this has yet to be proven.

Genetics of imipemide resistance. The plasmid profiles of a number of Imi^{s}, Imi^{dz}, and Imi^{r} isolates were examined using cultures of 2 to 5 ml and a variety of plasmid isolation procedures (data not shown). Each isolate examined contained four to eight plasmids detectable by UV illumination of ethidium bromide-stained agarose gels. The isolates could be clearly grouped by plasmid profile, but these groupings bore no relationship to their resistance or susceptibility to imipemide.

Attempts were made to "cure" Imi^{dz} or Imi^{r} isolates of a putative imipemide resistance plasmid. These attempts involved growing the isolates at 42°C or in the presence of acridine orange, ethidium bromide, or both. Some Imi^{s} derivatives were isolated that have not been found to revert to imipemide resistance, but the rate of their appearance was not measurably increased by any of the conditions listed above. The plasmid profiles of these Imi^{s} derivatives appeared to be identical to those of the parent strains, and some of the derivatives may have resulted from the mutagenic action of ethidium bromide and acridine orange.

The coagulase-negative staphylococci have considerable clinical significance in their own right (16, 22, 26, 43). Their importance may also extend to acting as a conduit for genes between

the soil microorganisms (27) and a wide range of other organisms, possibly including *Bacillus subtilis* (10), *Streptococcus pneumoniae* (1), *E. coli* (1), other staphylococci (4, 18), and even *Saccharomyces cerevisiae* (11). The mechanism, regulation, and epidemiology of imipemide resistance in the coagulase-negative staphylococci have yet to be clearly established. The results of such studies may well indicate the means by which an antibiotic resistance gene moves into clinically significant microorganisms before the industrial production and use of the relevant antibiotic.

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