

Antibiotic Resistance of Clinical Isolates of *Enterococcus* in Italy

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Alarming reports from various parts of the world of an increase in multiple-drug resistance among enterococci have prompted the Italian Association of Clinical Microbiologists to sponsor a nationwide study aimed at assessing the magnitude of this problem in Italy. During the last 3 months of 1988, technicians from a total of 162 general and teaching hospitals, in 16 of the 20 Italian regions and covering 89,061 beds, isolated 58,886 bacterial strains from inpatients and outpatients. Enterococci (4,879) represented 8.3% of all microorganisms identified, with *Enterococcus faecalis* accounting for 4,554 strains and *Enterococcus faecium* for the remaining 316 (6.5% of all enterococci analyzed). Enterococci were recovered most frequently from urine (62.5%) and genitourinary samples (13.7%). Only 58 strains (1.2%) were associated with bacteremia. The *in vitro* susceptibility of these organisms to 21 commonly used antibiotics is reported and compared with the susceptibility of isolates from a smaller epidemiologic study conducted in Italy in 1986 as well as with more recent findings from individual laboratories.

In the last several years, the prevalence of infections caused by gram-positive microorganisms has increased in the community as well as in nosocomial settings worldwide [1]. This ominous epidemiological trend may be the consequence of predisposing risk factors such as immunosuppression, instrumentation, long-term hospitalization, and wide use of broad-spectrum antibiotics, notably third-generation cephalosporins, which possess intrinsically moderate activity against these pathogens. Among the important gram-positive bacteria, the enterococci are a recognized cause of urinary, soft-tissue, intraabdominal, and wound infections [2]. Enterococcal bacteremia with or without endocarditis and meningitis also are becoming a frequent problem in tertiary hospitals [3, 4]. Therapeutic options for severe enterococcal infections may be limited, since these pathogens can be killed only with the use of a synergistic combination of a cell wall-active agent and an aminoglycoside [5]. Recent reports have indicated, however, that the enterococci, already known to be relatively unaffected by a variety of antibiotics, are being increasingly colonized by resistance plasmids that blunt the clinical efficacy of first-line agents such as β -lactam drugs [6], aminoglycosides [7], and glycopeptides [8]. In view of these alarming new trends and with the aim of assessing the magnitude of the problem in this country, we analyzed in detail and report here data concerning the distribution and prevalence of resistance to several antibiotics among clinical enterococcal strains isolated in Italy that have emerged from a nationwide study sponsored by the Italian Association of Clinical Microbiologists in 1988.

Materials and Methods

From October to December 1988, a total of 162 laboratories dispersed throughout the country (figure 1) that were monitoring bacteriologic isolates obtained from inpatients and outpatients were enrolled in the Italian Association of Clinical Microbiologists epidemiological survey. Most of the participating hospitals were acute care general hospitals. Of these, 92 had <500 beds, 45 had 500–1,000 beds, and 25 had >1,000 beds. The total number of beds was 89,061. Because of the large number of subscribing institutions, no strict experimental protocol was enforced. Instead, participants were requested to file data on all strains subjected to antibiotic susceptibility testing by the standard procedures operative in each center. As a result, bacteria were identified by several commercially available methods and sensitivity testing was performed by various techniques, including the manual Bauer-Kirby test (42% of all laboratories) and semiautomated and automated systems [9]. Test methods and materials differed widely among centers. Moreover, available reports could not document the existence of effective quality controls. Invaluable assistance in the processing of data was provided by Becton-Dickinson Italia (Milan).

Results

A total of 56,041 clinical specimens were processed by the 262 laboratories during the 3-month survey period. Of these, 30,420 (54.3%) were urine specimens. Throat swabs, pus and wound swabs, and sputum accounted for 8.7%, 6.2%, and 5.2% of the specimens, respectively. Only 2.6% of specimens were blood. From these samples, 58,886 bacteria deemed clinically significant were isolated, identified by species, and tested for antibiotic sensitivity. Of all organisms tested, Enterobacteriaceae accounted for 49.2%. *Escherichia coli* was the most frequently isolated organism (29.5%). *Staphylococ-*

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Figure 1. Distribution of Italian centers participating in the 1988 epidemiological survey of the Italian Association of Clinical Microbiologists. Figures indicate the number of centers in each region. Four regions (Val d' Aosta, Trentino-Alto Adige, Basilicata, and Sardinia) were not represented.

cus, *Pseudomonas*, *Enterococcus*, and *Streptococcus* accounted for 17.5%, 9.4%, 8.3%, and 7.5% of clinical strains, respectively, with other bacteria constituting the remaining 8.1%. Among the enterococci, there were 4,554 strains of *Enterococcus faecalis* and 316 strains of *Enterococcus faecium*. Table 1 lists the distribution of the 4,870 enterococcal isolates according to clinical source. Enterococci were recovered most frequently from urine, the genitourinary tract, wounds, and abscesses. These organisms were more often obtained from inpatients (64.1%) than from outpatients (35.9%) and were most prevalent in surgical departments. Overall, enterococci accounted for 23.5% of all gram-positive pathogens, ranking second in frequency to the staphylococci.

The *in vitro* susceptibilities of *E. faecalis* and *E. faecium* to 21 antibiotics commonly tested in clinical microbiology laboratories are shown in tables 2 and 3. Only truly resistant strains (i.e., those resistant by the Kirby-Bauer disk susceptibility test) were considered to be resistant. Moderately susceptible organisms were scored separately so as not to overestimate the levels of resistance. Given the open nature of the study protocol, repetitive isolates may have contributed, although unpredictably, to the susceptibility figures. Except for teicoplanin, which was introduced in Italy during 1988,

no data on antimicrobial drugs tested against <1,000 isolates were included in table 2.

Among pertinent findings are the presence of widespread resistance to penicillin in enterococci and the adequate performance by broad-spectrum penicillins *in vitro*. Excluding cephalosporins, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMZ)—agents that have little clinical efficacy against the enterococci but that were tested in this study largely because of their significant activity against other gram-positive pathogens [2]—most other “old” agents (tetracycline, erythromycin, clindamycin) were found to encounter diffuse resistance. The results obtained with all the aminoglycosides with tests in which standard disks or drug concentrations were used [9] confirm the intrinsic indifference of the enterococci to these agents and should not be considered to represent the prevalence of high-level resistance (>200 µg/mL) in Italy, an issue of the utmost clinical importance [2]. Rifampin remains active against >70% of isolates, and nitrofurantoin and amoxicillin/clavulanate (Augmentin) appeared highly efficacious *in vitro*. Glycopeptides (vancomycin and teicoplanin) were confirmed to be the most potent antienterococcal drugs [10–12]. As expected [2], in *E. faecium* the prevalence of resistance was higher with all antimicrobial agents tested (table 3) except teicoplanin. Comparison of the susceptibilities of the 58 *E. faecalis* isolates with those of the 2,737 urinary strains (data not shown) revealed that the pathogens isolated from blood were distinctly more susceptible to all the agents used in therapy. None of these organisms was either resistant or only moderately susceptible to vancomycin or teicoplanin. The same general trend was noted when resistance patterns of enterococci isolated from outpatients were compared with those from inpatients (data not shown).

Discussion

The data gathered in this survey, while representing the outcome of a remarkable cooperative effort, have several in-

Table 1. Clinical enterococcal isolates (*n* = 4,870).

Type of specimen	No. of enterococci isolated from specimen	No. (%) of isolates
Urine	2,737	56.2 (62.5)*
Vaginal swabs	416	8.5
Urine (catheter)	307	6.3
Pus	258	5.3
Urethral swabs	142	2.9
Semen	113	2.3
Throat swabs	103	2.1
Blood	58	1.2†
Other	736	15.2

* Number in parentheses is the percentage of isolates from urine and urinary catheters.

† Fifty-eight (3.9%) of 1,472 strains were isolated from blood cultures.

Table 2. Susceptibility of 4,554 strains of *E. faecalis* to 21 antimicrobial agents.

Antimicrobial agent	No. of strains tested	Percentage of strains		
		Susceptible	Moderately susceptible	Resistant
Penicillin	3,497	11	50	39
Ampicillin	4,353	72	20	8
Piperacillin	2,345	90	6	4
Mezlocillin	1,735	95	1	4
Amoxicillin/clavulanate	1,166	97	1	2
Cephalothin	3,057	8	23	69
Ceftriaxone	1,307	10	17	73
Imipenem	1,309	84	4	12
Tetracycline	3,261	36	5	59
Erythromycin	3,935	40	21	39
Clindamycin	4,080	7	3	90
Chloramphenicol	2,734	51	10	39
Trimethoprim-sulfamethoxazole	2,907	52	3	45
Gentamicin	2,716	18	12	70
Netilmicin	1,029	47	12	41
Amikacin	1,162	11	11	78
Rifampin	1,894	70	17	13
Vancomycin	2,770	96	3	1
Teicoplanin	875	97	2	1
Norfloxacin	2,378	62	20	18
Nitrofurantoin	2,877	95	2	3

trinsic limitations that must not be overlooked. The exact prevalence of enterococci cannot be inferred from present results since the survey included only those microorganisms selected for antimicrobial susceptibility testing. The prevalence of mixed infections cannot be extracted from the pooled data, a limitation that is particularly relevant for enterococci [2]. Furthermore, because of the lack of homogeneity of the testing procedures, the accuracy of the figures concerning resistance or susceptibility is open to criticism. This may render the task of comparing the present data with data reported in the literature, studies frequently plagued by similar flaws, extremely difficult.

Nevertheless, this survey fully confirms the threat posed today by gram-positive organisms in community- and hospital-acquired infections. In fact, gram-positive strains account for 35.3% of all pathogens isolated in Italy. In this study, the prevalence of the enterococci was 8.3%. In a previous tetracycline study conducted in 1986, enterococcal species accounted for 13.9% of the 4,359 microorganisms isolated [13]. In our own laboratories, during 1989 enterococci accounted for 16% of all bacteria identified (data not shown). Wide variations in prevalence in different centers can therefore be expected, possibly in response to the specific interest posed by the biology of a particular pathogen. When all Italian data are pooled, the prevalence of enterococci is 8.8%, a figure not strikingly different from that put forth by Atkinson and Lorian [14] in a much larger survey conducted in the United States. The distribution of *E. faecalis* and *E. faecium* in clini-

cal specimens and their respective prevalence in this study is in accordance with the habitats of these organisms and the spectrum of infections produced in humans [2].

In some European regions, until recently full susceptibility to β -lactam antibiotics, notably to penicillin and ampicillin, was considered to be a stable trait of *E. faecalis* and, to a lesser extent, of *E. faecium* [15–17]. On the contrary, Atkinson and Lorian [14] pointed out in 1984 that the prevalence of resistance to penicillin was 64% and increasing, while resistance to ampicillin had leveled off at 6.2% in *E. faecalis*. Our results indicate that resistance is widespread in the enterococci (39%–51%) and that ampicillin is inactive in vitro against 8% of *E. faecalis* and 18% of *E. faecium* isolates. Piperacillin and mezlocillin are uniformly more active than ampicillin against *E. faecalis* and *E. faecium*. Although these figures do not include the moderately susceptible strains, one interesting finding of this study is the low level of resistance met by amoxicillin/clavulanate, a suicide inhibitor of bacterial β -lactamases [18]. Clarification of the meaning of this finding awaits more direct investigations.

β -Lactam agents alone or in combination with other drugs are the mainstay of therapy for enterococcal infections. Therefore, the finding that ampicillin resistance in *E. faecalis* has increased to 8% in Italy, when it was reported to be 1% in 1984 [13], is particularly disturbing and is reminiscent of a trend that has parallels elsewhere [19]. In light of these findings, it seems mandatory to investigate the nature of the mechanisms that endow local enterococci with this trait and

Table 3. Susceptibility of 316 strains of *E. faecium* to 21 antimicrobial agents.

Antimicrobial agent	No. of strains tested	Percentage of strains		
		Susceptible	Moderately susceptible	Resistant
Penicillin	212	19	30	51
Ampicillin	262	67	15	18
Piperacillin	147	67	21	12
Mezlocillin	111	80	7	13
Amoxicillin/clavulanate	105	85	10	5
Cephalothin	188	21	16	63
Ceftriaxone	102	11	7	82
Imipenem	103	34	11	55
Tetracycline	235	42	7	51
Erythromycin	289	47	13	40
Clindamycin	210	19	8	73
Chloramphenicol	243	70	8	22
Trimethoprim-sulfamethoxazole	191	38	4	58
Gentamicin	193	18	15	67
Netilmicin	120	40	11	49
Amikacin	99	14	36	50
Rifampin	162	59	14	27
Vancomycin	206	96	2	2
Teicoplanin	120	98	1	1
Norfloxacin	174	43	20	37
Nitrofurantoin	180	71	8	21

to determine whether altered penicillin-binding proteins or transmissible plasmids coding for a β -lactamase are the sources of this resistance problem [6, 20–22].

Because disk diffusion tests do not discriminate between the natural moderate resistance of the enterococci to aminoglycosides and the clinically relevant high-level resistance, the data gathered in the survey are of minor interest in interpreting the response of these pathogens to these drugs. However, a recent review points out that, in at least some Italian settings the incidence of high-level resistance to gentamicin increased from 5% in 1985 to 8.4% in 1986 [23]. Our country, therefore, is not immune to the rapid dissemination of plasmids containing resistance determinants that prevent synergistic killing of enterococci by the combination of aminoglycosides with cell wall-active agents [16, 24, 25].

If one arbitrarily assumes that an antimicrobial drug should not encounter a resistance rate of >10% for it to be used empirically for the treatment of common infections and yield a high probability of a favorable (90%) outcome under the conditions prevailing in family practice—for which etiologic diagnosis is rarely available—the empiric regimens one would recommend according to the findings of this survey are nitrofurantoin for urinary tract infections sustained by *E. faecalis* and vancomycin and teicoplanin for all other enterococcal infections.

While clinical laboratories that use disk diffusion techniques may have failed to categorize as resistant those enterococcal strains that have reduced susceptibility to vancomycin

[26], this and previous studies [11, 12, 27] clearly confirm that presently the glycopeptides are the agents active against gram-positive organisms that are least plagued by resistance problems. The magnitude of the biological and clinical threats posed by the emergence of enterococci that harbor plasmids conferring resistance to these drugs [8, 28, 29] remain to be established and warrant close monitoring.

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