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Antimicrobial susceptibility patterns of *Staphylococcus aureus* in Poland obtained by the National Quality Assurance Programme

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ABSTRACT

As part of the Polish external quality assurance scheme, clinical laboratories were asked to send five consecutive isolates of *Staphylococcus aureus* and the corresponding susceptibility results to the national Centre of Quality Control in Microbiology. Of 1376 isolates submitted as *S. aureus* from 276 medical centres, 13 (< 1%) had been misidentified by local laboratories. Of 181 (13.5%) methicillin-resistant *S. aureus* (MRSA) isolates, most were identified correctly (*c.* 98% of laboratories). Although all MRSA isolates were fully susceptible to vancomycin, teicoplanin and linezolid, they were usually multiresistant; almost 23% were resistant to seven antimicrobial agents. Most (> 90%) MSSA isolates were susceptible to the tested antibiotics, except penicillin (21% susceptible) and tetracycline (62.4% susceptible). In addition to evaluating the proficiency of testing by local laboratories, the study yielded valuable information regarding the susceptibility patterns of *S. aureus* isolates in Poland.

Keywords Antimicrobial susceptibility, MRSA, Poland, proficiency testing, *Staphylococcus aureus*, surveillance

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INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens, responsible for a variety of infections in all age groups. It is also challenging to treat because of its resistance to antimicrobial agents. In addition to universal β-lactamase production, *S. aureus* isolates resistant to methicillin (MRSA), and thus resistant to all β-lactam antibiotics, have spread worldwide and are responsible for nosocomial and community outbreaks of infection [1–3]. In addition, MRSA isolates with either reduced susceptibility or high-level resistance to vancomycin have now been described [4,5].

Accurate determination of resistance phenotype and the underlying mechanisms of resistance are of crucial importance, not only for therapy, but also from a public health perspective. In addition to internal laboratory quality control procedures, several national and international external quality control assurance schemes for antimicrobial susceptibility testing have been established in order to assess the proficiency of testing in individual laboratories and to compare laboratories within a country or on an international level [6–10]. The Polish external quality assurance scheme (POLMICRO) was established in 1994 and, since 1997, has been coordinated by the Centre of Quality Control in Microbiology (CQCM). In the present study, participating laboratories were asked to send *S. aureus* isolates, with the corresponding susceptibility results, to the CQCM. The isolates were re-identified and MICs of a broad panel of antibiotics were determined by the CQCM in order to evaluate the testing proficiency of the local laboratories and to gather *S. aureus* susceptibility data from throughout Poland.

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MATERIALS AND METHODS

Study design

Local laboratories

Laboratories (n = 276) participating in the POLMICRO scheme sent five consecutive clinical isolates of *S. aureus* (one isolate/patient) (n = 1376) during a 3-month period in 1999–2000 to the CQCM. Data on susceptibility to antimicrobial

agents (obtained by disk diffusion tests) and additional information (e.g., hospital or ambulatory care, type of ward, site of infection) were collected.

Centre of Quality Control in Microbiology

Most (n = 1005; 73.7%) of the *S. aureus* isolates were from hospital-acquired infections, including skin and soft tissue infections (n = 685; 50.2%), deep abscesses (n = 121; 8.9%), blood (n = 103; 7.6%), and bone and joint infections (n = 90; 6.6%). Isolates were collected from surgical (n = 388), internal medicine (n = 96), intensive care (n = 53), obstetric and gynaecology (n = 56), paediatric (n = 38), dermatology (n = 37), neonatology (n = 32) and other unspecified wards. Most community-derived isolates were recovered from skin and soft tissue infections. The isolates were re-identified by standard procedures [11], based on free coagulase production and clumping factor (rabbit plasma; Biomed, Warsaw, Poland) and DNase production (DNase agar; Mast Diagnostics, Bootle, UK).

Susceptibility testing

Methicillin resistance was determined by disk diffusion with a 1-μg oxacillin disk, an oxacillin agar screening test (performed when discrepancies between local laboratory and CQCM results were encountered) [12,13] and detection of the *mecA* gene by PCR (performed on all MRSA isolates identified by oxacillin disk diffusion) [14]. *S. aureus* ATCC 29213 (methicillin-susceptible, MSSA) and *S. aureus* ATCC 43300 (methicillin-resistant) were included as reference strains.

Antimicrobial susceptibility testing was performed by both disk diffusion and agar dilution methods according to NCCLS guidelines [12,13]. The following antimicrobial agents were used for disk diffusion tests by local laboratories: penicillin, oxacillin, erythromycin, clindamycin, lincomycin, gentamicin, tetracycline, doxycycline, ciprofloxacin, ofloxacin, trimethoprim–sulphamethoxazole, teicoplanin, vancomycin, rifampicin, chloramphenicol, nitrofurantoin, fusidic acid (Becton Dickinson, Franklin Lakes, NJ, USA) and mupirocin (Oxoid, Basingstoke, UK). *S. aureus* ATCC 25923 (methicillin-susceptible) was used as the control strain for disk diffusion testing.

MICs were determined for 19 antibiotics (MRSA isolates) and 24 antibiotics (MSSA isolates) by agar dilution on Mueller–Hinton-II Agar (Becton Dickinson). An inoculum of 10^4 CFU was applied to antibiotic-containing plates with a multipoint inoculator (West Sussex Instruments Ltd, Denley, UK). The following antimicrobial agents were tested: penicillin (Sigma, Munich, Germany), cloxacillin (Polfa Tarchomin, Warsaw, Poland), amoxycillin–clavulanic acid (SmithKline Beecham, Philadelphia, PA, USA), cefazolin (Fluka, Buchs, Switzerland), cefuroxime (Sigma), erythromycin (Fluka), clindamycin (Pharmacia Upjohn, Kalamazoo, MI, USA), gentamicin (Polfa Tarchomin), tetracycline (Sigma), doxycycline (Sigma), minocycline (Wyeth Ayerst, St Davids, PA, USA), ciprofloxacin (KRKA, Novo Mesto, Slovenia), moxifloxacin (Bayer, Wuppertal, Germany), trimethoprim–sulphamethoxazole (Roche, Basel, Switzerland), trimethoprim (Roche), teicoplanin (Marion Merrell, Denham, UK), vancomycin (Lilly, Indianapolis, IN, USA), rifampicin (Lepetit, Lainate, Italy), chloramphenicol (Sigma), linezolid (Pharmacia Upjohn), quinupristin–dalfopristin (Aventis Pharma, Romainville, France), nitrofurantoin (Terpol, Sieradz, Poland), fusidic acid (Aldrich, Taufkirchen, Germany) and mupirocin (SmithKline Beecham). Breakpoints for fusidic acid, mupirocin, cloxacillin and doxycycline were those recommended by Comité de l'Antibiogramme de la Societé Francaise de Microbiologie guidelines [15].

S. aureus ATCC 29213 (MSSA), *Escherichia coli* ATCC 35218 (for β-lactam/β-lactamase inhibitor combinations) and *Pseudomonas aeruginosa* ATCC 27853 (for Mueller–Hinton agar) were used for quality control of the susceptibility tests [13]. The inducible MLS_B ($iMLS_B$) phenotype was detected by the double erythromycin–clindamycin disk test.

RESULTS

Quality control data

Of 1376 isolates submitted as *S. aureus*, 13 (< 1%) were misidentified by local laboratories and comprised various coagulase-negative species, such as *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus xylosus* and *Staphylococcus hominis*. Of those isolates identified correctly as *S. aureus* (n = 1363), 27 (2.0%) were misclassified according to methicillin susceptibility, in that 25 were reported as MRSA instead of MSSA (a major error: no *mecA* gene detected) and two as MSSA instead of MRSA (a very major error). All MRSA isolates were reported correctly as resistant to all β-lactams. Interpretative errors detected in the testing of other antimicrobial agents are shown in Table 1. Inducible MLS_B resistance was not detected in one isolate by three laboratories, and in two isolates by one laboratory.

Table 1. Interpretative errors observed following susceptibility testing by disk diffusion

Test result → reference result (error type)	No. of laboratories					
	Erythromycin	Tetracycline	Ciprofloxacin	Trimethoprim–sulphamethoxazole	Clindamycin	Gentamicin
S → R (very major)	0	5	0	0	0	0
R → S (major)	4	5	4	7	2	2
S → I (minor)	0	0	0	0	0	0
I → S (minor)	11	2	6	2	5	4
R → I (minor)	0	0	0	0	0	0
I → R (minor)	0	0	0	0	0	0
Total	15	12	10	9	7	6

S, sensitive; R, resistant; I, intermediate.

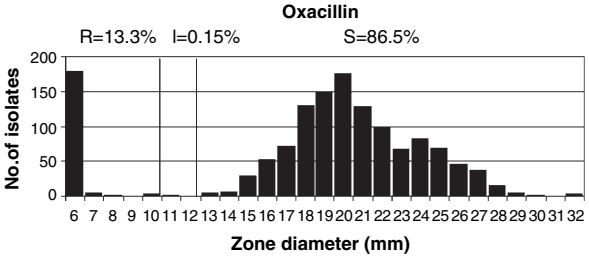

Fig. 1. Distribution of oxacillin disk zone diameters. S, susceptible; I, intermediate; R, resistant.

In the histograms of zone diameter distributions for oxacillin (Fig. 1) and other selected antimicrobial agents (Fig. 2), unimodal distribution was seen, indicating good separation of susceptible and resistant populations. The tetracycline resistance rate was extremely high (> 46%).

Antibiotic susceptibility results

Most (> 90%) MSSA isolates were susceptible to most antibiotics, with the exception of penicillin (21% susceptible) and tetracycline (62.4% susceptible) (Table 2). One *mecA*-negative isolate was resistant to cloxacillin and could represent the BORSA (borderline oxacillin-resistant *S. aureus*) phenotype. No differences in the susceptibility data were observed between hospital and community isolates of MSSA; thus, the data were combined. MRSA isolates were fully susceptible to vancomycin, teicoplanin and linezolid, compared to tetracycline (18% susceptible), gentamicin (25% susceptible), erythromycin (31% susceptible) and ciprofloxacin (42% susceptible) (Table 3). According to the MIC data, 51.1% were susceptible to clindamycin, but when the inducible mechanism was taken into consideration, only 34.3% were

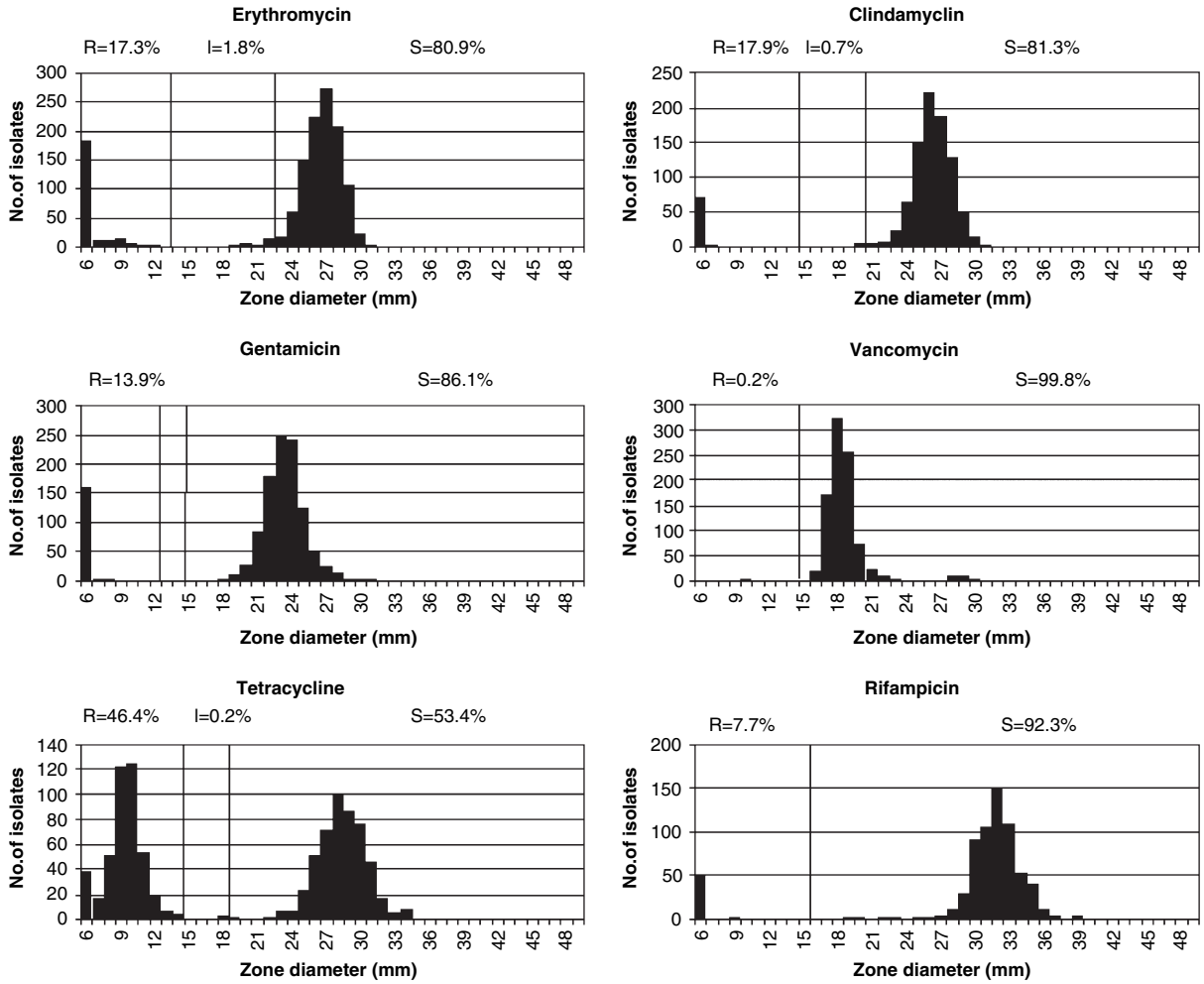

Fig. 2. Distribution of disk zone diameters for erythromycin, clindamycin, gentamicin, vancomycin, tetracycline and rifampicin for all tested isolates. S, susceptible; I, intermediate; R, resistant.

Table 2. Number of methicillin-susceptible *Staphylococcus aureus* isolates (n = 1182) with the indicated MICs of selected antimicrobial agents

	MIC (mg/L)																
Antimicrobial agent	**≤ 0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**MIC_{50}**	**MIC_{90}**	**S%**
Penicillin	5	87	100	54	150	434	288	47	16	1	–	–	–	–	0.5	1	21
Cloxacillin	–	4	29	395	646	106	1	–	–	1	–	–	–	–	0.25	0.25	99.9
Amoxycillin–clavulanic acid	–	15	19	179	329	578	61	1	–	–	–	–	–	–	0.5	0.5	100
Cefazolin	–	–	14	26	271	747	102	21	–	–	1	–	–	–	0.5	1	99.9
Cefuroxime	–	–	–	4	33	192	788	161	2	–	–	1	–	–	1	2	99.9
Erythromycin	–	–	5	64	841	162	4 (1[a])	3 (3[a])	5 (5[a])	5 (4[a])	4 (2[a])	10(4[a])	79 (43[a])	–	0.25	0.5	90.7
Clindamycin	–	34 (3[a])	568 (24[a])	456 (25[a])	96 (10[a])	1	–	–	–	1	26	–	–	–	0.06	0.25	97.8 (92.5[a])
Gentamicin	–	–	2	32	705	406	4	3	1	–	1	3	25	–	0.25	0.5	97.7
Tetracycline	–	3	21	239	403	66	1	3	2	17	68	210	127	22	0.25	64	62.4
Doxycycline	7	38	229	363	93	17	65	199	137	22	8	4	–	–	0.12	4	97.1
Minocycline	5	35	531	467	99	5	7	12	14	7	–	–	–	–	0.12	0.25	99.5
Ciprofloxacin	–	11	2	36	421	628	68	9	3	2	2	–	–	–	0.5	0.5	98.6
Moxifloxacin	65	313	632	162	6	1	–	2	1	–	–	–	–	–	0.06	0.12	99.7
Trimethoprim–sulphamethoxazole	12	187	329	180	359	77	7	2	9	7	3	10	–	–	0.12	0.25	97.5
Trimethoprim	–	–	–	5	29	304	624	165	29	3	6	4	4	9	1	2	98
Teicoplanin	–	–	7	10	82	564	512	7	–	–	–	–	–	–	0.5	1	100
Vancomycin	–	–	–	4	12	745	413	8	–	–	–	–	–	–	0.5	1	100
Rifampicin	1177	3	–	–	–	–	–	–	–	2	–	–	–	–	0.0075	0.015	99.8
Chloramphenicol	–	–	–	–	–	7	1	37	341	741	17	9	26	2	8	8	95.4
Linezolid	–	–	9	3	8	33	352	772	5	–	–	–	–	–	2	2	99.6
Quinupristin–dalfopristin	–	4	1	6	276	834	61	–	–	–	–	–	–	–	0.5	0.5	100
Nitrofurantoin	–	–	–	–	–	–	2	9	35	733	400	3	–	–	8	16	100
Fusidic acid	5	88	397	616	72	2	–	–	1	1	–	–	–	–	0.12	0.12	99.8
Mupirocin	4	11	264	763	109	1	–	–	–	1	29	–	–	–	0.12	0.25	97.4

S, susceptible.
[a]Inducible resistance phenotype MLS_B.

Table 3. Number of methicillin-resistant *Staphylococcus aureus* isolates (n = 181) with the indicated MICs of selected antimicrobial agents

	MIC (mg/L)																
Antimicrobial agent	**≤ 0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**MIC_{50}**	**MIC_{90}**	**S%**
Erythromycin	–	–	2	13	37	1	1 (1[a])	5 (5[a])	10 (8[a])	8 (6[a])	3	–	101 (9[a])	–	> 64	> 64	31.4
Clindamycin	–	3	28 (4[a])	51 (19[a])	8 (5[a])	–	–	–	–	–	–	91 (1[a])	–	–	0.25	> 32	51.1 (34.3[a])
Gentamicin	–	–	–	1	16	24	1	–	–	–	–	2	14	122	128	> 128	25
Tetracycline	–	1	2	22	3	1	–	–	1	5	33	46	23	44	32	128	18
Doxycycline	–	6	16	12	–	4	5	43	46	28	17	4	–	6	4	16	70.7
Minocycline	1	2	20	13	8	10	46	35	28	18	–	–	–	–	1	8	89.9
Ciprofloxacin	–	–	3	9	31	18	11	7	9	43	41	9	–	–	4	16	42
Moxifloxacin	–	17	33	23	5	3	21	58	19	2	–	–	–	–	1	4	58
Trimethoprim–sulphamethoxazole	2	3	27	41	36	17	15	9	7	4	2	12	–	–	0.25	4	86.7
Trimethoprim	–	–	–	7	22	39	53	29	1	5	3	–	1	21	1	128	86.7
Teicoplanin	–	–	2	9	36	58	33	33	9	1	–	–	–	–	0.5	2	100
Vancomycin	–	–	–	–	12	54	85	30	–	–	–	–	–	–	1	2	100
Rifampicin	106	3	–	–	–	–	–	1	1	–	1	69	–	–	0.015	> 32	61.7
Chloramphenicol	–	–	–	–	–	–	10	38	81	28	11	3	8	2	4	16	87.2
Linezolid	–	–	–	–	21	78	69	13	–	–	–	–	–	–	0.5	1	100
Quinupristin–dalfopristin	–	–	4	8	41	63	48	17	–	–	–	–	–	–	0.5	1	90.9
Nitrofurantoin	–	–	–	–	–	–	–	1	32	103	45	–	–	–	8	16	100
Fusidic acid	7	36	19	44	12	3	9	27	23	1	–	–	–	–	0.12	4	87.2
Mupirocin	1	14	94	57	6	–	–	–	1	–	7	–	–	1	0.06	0.12	95.7

S, susceptible.
[a]Inducible resistance phenotype MLS_B.

susceptible. However, susceptibility to erythromycin was not changed, since all isolates with the $iMLS_B$ phenotype fell into the intermediate category (data not shown). Almost 23% of the isolates exhibited resistance to seven different antimicrobial agents.

Prevalence of resistance

In total, 181 (13.3%) isolates were identified as methicillin-resistant (*mecA*-positive). Most (n = 177; 97.6%) were from hospitalised patients, originating from intensive care (45.3%), surgical

(17%), internal medicine (16.6%), paediatric (7.9%) and neonatology (6.3%) wards. MRSA isolation rates were highest from surgical site infections (21.9%), joint and bone infections (20.2%) and blood (19.6%) (Table 4). The prevalence of MRSA was higher in secondary- and tertiary-care institutions (15.3%) than in primary care (11.7%), and varied significantly between regions, being lowest in the eastern part of Poland (Fig. 3).

DISCUSSION

In an era of growing antibiotic resistance and the emergence and spread of multiresistant bacteria, the accurate detection of susceptibility phenotypes is crucial for patient management and for infection control practices. The most important mechanism of resistance in staphylococci is resistance to methicillin, which in clinical terms signifies resistance to all β-lactam antibiotics, and is often accompanied by resistance to many other groups of antimicrobial agents. In addition, clonal spread of MRSA occurs, with several international and local clones causing epidemics [2]. The recent appearance of community-acquired MRSA has underlined the importance of accurate detection of this resistance phenotype outside the hospital [3,16,17], and therefore many external quality control schemes, including POLMICRO, incorporate staphylococci in their programmes [6,7]. When the POLMICRO programme started in 1994, *c.* 50% of participating laboratories were unable to detect methicillin resistance [18]. The situation has started to improve as a result of detailed feedback provided by the CQCM, including updated methodology, detailed analysis of mistakes and problems encountered (http://www.polmikro.edu.pl), and provision of free training. In 1998, misidentification of resistance occurred in 30% of laboratories, decreasing to 12.5% in 2000 [19–21]. In the present study, most (*c.* 98%) laboratories identified MRSA correctly, and in POLMICRO 2002, <3% of laboratories failed to identify resistance correctly [22]. Accurate detection of methicillin resistance was achieved with the 1-μg oxacillin disk diffusion test, which appears to be a suitable diagnostic procedure for laboratories with limited resources. Only a small proportion of laboratories reported other antibiotic resistance phenotypes incorrectly. Since the establishment of POLMICRO, identification of *S. aureus* to the species level has not been problematic, as also reported by other external quality control schemes such as UKNEQAS and the CDC/WHO exercise [10]. In the present study <1% of isolates were misidentified.

Although the main purpose of the present study was to evaluate the ability of Polish microbiological laboratories to detect resistance phenotypes in clinical isolates of *S. aureus*, important additional information was also obtained regarding the incidence of MRSA throughout the country, and the prevalence of MRSA with regard to the site of infection and type of hospital ward affected. Although overall resistance among hospital isolates was 17.6%, it was interesting to observe differences between various regions of Poland, with the lowest

Table 4. Source of *Staphylococcus aureus* isolates from hospitalised patients

Sample type	Total (n = 1003)	MSSA (n = 826; 82.3%)	MRSA (n = 177; 17.6%)
Skin and soft tissue	284	240	44 (15.5%)
Surgical site infection	251	196	55 (21.9%)
Deep abscess	87	77	10 (11.5%)
Blood	102	82	20 (19.6%)
Joint and bone infection	74	59	15 (20.2%)
Otitis media	22	22	–
Conjunctivitis	17	17	–
Urinary tract infection	19	17	2 (10.5%)
Others	122	97	25 (20.5%)
Unknown	25	19	6 (24%)

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

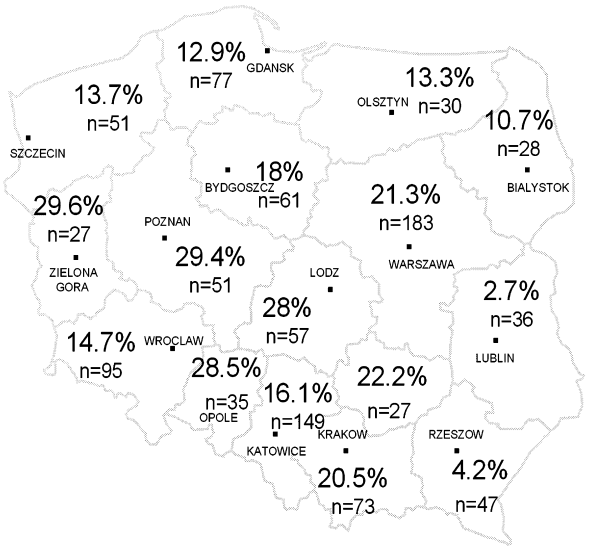

Fig. 3. MRSA prevalence (%) by regions of Poland. n = number of all hospital isolates from each region.

percentage of MRSA being found in the least industrialised, rural eastern area, where mainly primary-care facilities exist. Although the number of isolates/centre was low, the number of resistant isolates in each region was calculated from data from >30 laboratories. Pronounced geographical variations have also been reported previously in the prevalence of MRSA in different countries, and in different locations within a single country [2,23,24]. This emphasises the necessity of monitoring the local incidence of MRSA in order to optimise empirical therapy and infection control practices. With an overall frequency of 17.6%, Poland is in the middle of the range of MRSA incidence among European countries. For many years, the Scandinavian countries and The Netherlands have had the lowest incidence (1%), and Greece, Italy and France have had some of the highest incidences (> 40%) [25,26]. A significant increase in the incidence of MRSA has also been observed recently among isolates of *S. aureus* recovered in the UK [25,27]. Polish MRSA isolates are multiresistant, being fully susceptible only to glycopeptides and linezolid (personal unpublished data) [24,28,29]. No isolates with lowered susceptibility to glycopeptide antibiotics were identified in the present study, although the first such isolates have been detected in Poland [30]. Most MRSA isolates from Poland are susceptible to minocycline [31], which has never been registered for use in Poland. The highest proportion of MRSA isolates was noted in intensive care units, underlining the necessity for strict infection control measures in the care of critically ill patients. In contrast, MSSA isolates from nosocomial and community-acquired infections were mostly susceptible to all the antimicrobial agents tested except tetracycline, which may be the result of the high consumption of this drug in Poland for many years. However, it should be stressed that the presence of the inducible resistance phenotype should be investigated when testing susceptibility to macrolides and lincosamides, since MIC data underestimate the percentage of non-susceptible isolates.

In contrast to other countries, the present study identified a low susceptibility of MRSA isolates to rifampicin in Poland, although similar results have been obtained in some regions of Australia [32–34]. Resistance may arise in tuberculosis patients treated with rifampicin [35], and rifampicin is used extensively in Poland as part of combination therapy for tuberculosis, which has a relatively high incidence in the Polish population (26.5/100 000; http://www.pzh.gov.pl).

Overall, the present study emphasised that changing patterns of resistance among clinically important bacterial pathogens require careful monitoring and a consistently high quality of hospital laboratory testing.

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