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Short Communication

Virulence and antimicrobial resistance of *Staphylococcus aureus* isolated from bloodstream infections and pneumonia in Southern Poland

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ABSTRACT

Objectives: Staphylococcus aureus remains the most important cause of infections in hospitals and long-term care facilities. The aim of this study was to analyse the resistance, virulence, and epidemiological and genetic relationships of *S. aureus* from bloodstream infections (BSIs) and pneumonia from patients in Southern Poland.

Methods: All strains were tested for antimicrobial susceptibility using the disk diffusion method. Etest was also performed for vancomycin, teicoplanin, tigecycline, oxacillin, cefoxitin and penicillin. PCR amplification was used to detect selected virulence genes. The genetic similarity of methicillin-resistant *S. aureus* (MRSA) isolates was determined by *spa* typing and pulsed-field gel electrophoresis (PFGE). Using the BURP algorithm and the Ridom SpaServer database, *spa* types were clustered into different clonal complexes (spa-CCs).

Results and conclusions: MRSA strains were observed at a prevalence of 26.7%, but 88.6% of hospital-acquired infections were MRSA, with no difference between BSIs and pneumonia. The highest resistance was observed to erythromycin and tobramycin. None of the strains were resistant to linezolid, glycopeptides or tigecycline. The strains had no significant virulence factors and the number of virulence genes present did not correlate with the degree of drug resistance. PFGE typing showed relatively high diversity of strains. The majority of isolates belonged to *spa* type t003 (CC5).

1. Introduction

Staphylococcus aureus is one of the most important micro-organisms of the human commensal flora and is reported in 58% of hospitalised patients or residents of long-term care facilities (LTCFs) [1]. *S. aureus* remains one of the most important causes of infections in hospitalised patients and LTCF residents and usually represents a large part of the aetiology of infections. In the USA, the US Centers for Disease Control and Prevention (CDC) reported a prevalence of *S. aureus* in hospital infections of ca. 18% [2]. From a hospital epidemiology and surveillance of infections perspective, this pathogen remains the most important cause of bloodstream infections (BSIs) and pneumonia. Furthermore methicillin-resistant *S. aureus* (MRSA) is frequently isolated, not only in healthcare-associated infections. Strains classified as community-associated MRSA usually remain susceptible to majority of antibiotics, excluding β-lactams [3]. A recent report from the European Centre for Disease Prevention and Control

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(ECDC) showed large variations between European countries in 2015 in MRSA percentages, ranging from 0% to 57.2% among invasive *S. aureus* isolates. For Poland, among all invasive *S. aureus* isolates reported in 2015, 15.8% were MRSA [4].

Despite many reports regarding MRSA isolates among Polish patients, little is known about the molecular epidemiology of *S. aureus* causing invasive infections (both BSI and pneumonia) in Southern Poland. The aim of this study was to analyse the resistance, virulence, and epidemiological and genetic relationships of *S. aureus* from BSIs or pneumonia from hospitalised patients in Southern Poland.

2. Materials and methods

For this laboratory-based, multicentre study, samples were collected in collaboration with two laboratories between 1 January 2013 and 31 December 2013. Non-duplicate samples from 49 cases of BSI and 52 case of pneumonia were collected from hospitalised adult patients of hospitals and LTCFs throughout the south of Poland. The study included 13 hospitals (labelled with letters A–M; teaching hospitals were denoted by K and M), in which there were a total of 3362 beds (median, 195 beds; interquartile range, 154.5–300.5 beds). Relevant patient information, including age, sex and place of treatment [intensive care unit (ICU) or other wards (non-ICU)] was also collected. BSI and pneumonia was defined in accordance with the ECDC definition (https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/healthcare-associated-infections-HAI-ICU-protocol.pdf; accessed 26 September 2017). Screening for MRSA and eradication based on strictly established rules prior to hospitalisation were not routinely performed in any of the hospitals included.

All strains were tested for antimicrobial susceptibility by the disk diffusion method according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/clinical_breakpoints/; accessed December 2015). Antimicrobial disks were obtained from Oxoid Ltd. (Basingstoke, UK). Etest was also performed for vancomycin, teicoplanin, tigecycline, oxacillin, cefoxitin and penicillin (bioMérieux, Paris, France). The macrolide–lincosamide–streptogramin B (MLS_B) resistance phenotype of the isolates was determined according to a previously published protocol [5]. Total DNA was isolated from bacterial strains using a Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland). The MRSA phenotype was confirmed in all strains by detection of the *mecA* gene by PCR amplification using previously published primers [6]. *S. aureus* ATCC 33591 was used as a positive control. PCR amplification was used to detect the selected virulence genes: *lukE* (LukE leukocidin); *pvl* (PVL, Panton–Valentine leukocidin); *tsst-1* (TSST-1, toxic shock syndrome toxin-1); and *etA* and *etB* (ETA and ETB, exfoliative toxin A or B) [7].

To determine the *spa* type of the polymorphic X-region of the *S. aureus* protein A, the *spa* gene was amplified by PCR and was sequenced.

Chromatograms obtained from sequencing were analysed using Ridom StaphType software v.2.2.1 (Ridom GmbH, Würzburg, Germany; http://spa.ridom.de/index.shtml) to determine the *spa* type of each isolate [8]. Using the BURP algorithm (Ridom GmbH) and the Ridom SpaServer database [9], *spa* types were clustered into different clonal complexes (spa-CCs), and multilocus sequence typing (MLST) clonal complexes (CCs) were inferred. A singleton was defined as a *spa* type that was not grouped into a clonal complex.

The process of conducting the analysis of genetic similarity of the MRSA isolates was performed by pulsed-field gel electrophoresis (PFGE) in accordance with a previously published protocol [10]. Isolates that clustered ≥95% were considered as epidemic clones.

Categorical data were compared using Fisher's exact test. In addition, proportions of categorical variables (virulence factors and antimicrobial resistance) were reported. Statistical analysis was performed using Statistica 10 software (StatSoft, Inc., Tulsa, OK). *P*-values of ≤0.05 were considered to be statistically significant.

3. Results

The median age of the patients was 61 years in BSI cases and 60 years in pneumonia cases. Male patients accounted for significant part of the group, especially in BSI (37/49; 75.5%). Most of the observed infections were hospital-acquired infections (HAIs) (88/101; 87.1%) and only HAIs were registered in the ICU. An MRSA phenotype was detected in 27 *S. aureus* strains (26.7%) (Table 1) and was confirmed by the presence of the *mecA* gene.

Most cases of invasive infections were associated with patients treated in non-ICU wards (76/101; 75.2%). The prevalence of MRSA in BSI was 20.4% (10/49), but in pneumonia is was almost significantly higher [32.7% (17/52); $P = 0.0568$]. Among HAIs, 88.6% of strains were MRSA, with no difference between BSIs and pneumonia (Table 1).

An MLS_B phenotype was observed in 28.7% of strains (29/101) (Table 1).

The highest resistance was observed to erythromycin and tobramycin. None of the strains were resistant to linezolid, glycopeptides or tigecycline. Overall, analysis of drug sensitivity

Table 1
Comparison between bloodstream infections (BSIs) and pneumonia (PNU), and between infections treated in intensive care unit (ICU) and non-ICU wards.

	BSI (*N* = 49) *n* (%)	PNU (*N* = 52) *n* (%)	OR (95% CI)	*P*-value	ICU (*N* = 25) *n* (%)	Non-ICU (*N* = 76) *n* (%)	OR (95% CI)	*P*-value
Age (years) [median (IQR)]	61 [51–75]	60 [38–72]	n/a	0.6	60 [52–76]	60 [38–75]	n/a	0.6
Sex								
Male (*N* = 64)	37 (75.5)	27 (51.9)	2.9 (1.22–6.67)	0.01	15 (60.0)	49 (64.5)	0.8 (0.33–2.09)	0.9
Female (*N* = 37)	12 (24.5)	25 (48.1)			10 (40.0)	27 (35.5)		
HAI								
No (*N* = 13)	9 (18.4)	4 (7.7)	2.7 (0.77–9.43)	0.2	0 (0.0)	13 (17.1)	n/a	0.0343
Yes (*N* = 88)	40 (81.6)	48 (92.3)			25 (100.0)	63 (82.9)		
MRSA								
No (*N* = 74)	39 (79.6)	35 (67.3)	2.5 (0.96–6.46)	0.0568	17 (68.0)	57 (75.0)	0.7 (0.26–1.90)	0.7
Yes (*N* = 27)	10 (20.4)	17 (32.7)			8 (32.0)	19 (25.0)		
MLS_B								
No (*N* = 72)	35 (71.4)	37 (71.2)	1.0 (0.43–2.40)	0.9	16 (64.0)	56 (73.7)	0.6 (0.24–1.66)	0.5005
Yes (*N* = 29)	14 (28.6)	15 (28.8)			9 (36.0)	20 (26.3)		

OR, odds ratio; CI, confidence interval; IQR, interquartile range; n/a, not applicable; HAI, hospital-acquired infection; MRSA, methicillin-resistant *Staphylococcus aureus*; MLS_B, macrolide–lincosamide–streptogramin B mechanism.

Table 2
Number (%) of resistant strains in intensive care unit (ICU) and non-ICU wards.

Antimicrobial agent	Total (*N* = 101)	ICU (*N* = 25)	Non-ICU (*N* = 76)	OR (95% CI)
Cefoxitin	27 (26.7)	8 (32.0)	19 (25.0)	1.4 (0.53–3.79)
Ciprofloxacin	28 (27.7)	8 (32.0)	20 (26.3)	1.3 (0.49–3.52)
Moxifloxacin	26 (25.7)	8 (32.0)	18 (23.7)	1.5 (0.56–4.09)
Amikacin	28 (27.7)	11 (44.0)	17 (22.4)	2.7 (1.00–7.1)
Gentamicin	15 (14.9)	7 (28.0)	8 (10.5)	3.8 (1.19–12.34)
Netilmicin	15 (14.9)	7 (28.0)	8 (10.5)	3.3 (1.1–10.30)
Tobramycin	29 (28.7)	11 (44.0)	18 (23.7)	2.5 (1.00–6.50)
Erythromycin	29 (28.7)	9 (36.0)	20 (26.3)	1.6 (0.60–4.13)
Clindamycin	20 (19.8)	7 (28.0)	13 (17.1)	1.9 (0.65–5.43)
SXT	9 (8.9)	5 (20.0)	4 (5.3)	4.5 (1.10–18.3)

OR, odds ratio; CI, confidence interval; SXT, trimethoprim/sulfamethoxazole.

revealed that strains isolated from non-ICU *S. aureus* were frequently susceptible to antibiotics [especially gentamicin, netilmicin and trimethoprim/sulfamethoxazole (SXT)] (Table 2). The MIC_{50}/MIC_{90} values (minimum inhibitory concentrations for 50% and 90% of the isolates, respectively) for teicoplanin was 2.0/3.0 mg/L in BSI cases and 1.5/2.2 mg/L in pneumonia cases. There were no differences between MICs in ICU and non-ICU strains. The MICs for oxacillin, cefoxitin and penicillin for all MRSA strains are shown in Fig. 1).

In the observed group of micro-organisms, *lukE* was the most frequently observed virulence gene (72.3%; 73/101), whereas *pvl*, *eta* and *tsst* genes were present only in methicillin-susceptible *S. aureus* (MSSA) strains ($n = 2, 4$ and 4, respectively). The number of virulence genes present in strains did not correlate with the degree of drug resistance (MSSA vs. MRSA, $P = 0.07$).

Analysis of *Sma*I macrorestriction profiles of MRSA isolates did not revealed a dominant clone. The 27 MRSA isolates showed 20 pulsotypes that were <90% similar. Strains with identical pulsotypes (mainly from hospitals K, M and B) are shown in Fig. 1.

spa typing of 27 MRSA isolates showed the presence of 10 different *spa* types (Fig. 1), with two novel types not identified previously (t17029 and t17030). *spa* type t003 dominated (13 strains; 48.1%) and was detected in 7 of the 13 hospitals (Table 3).

Using the Ridom StaphType software v.2.2.1 [8], 10 *spa* types were distributed by the BURP algorithm (with the calculated cost between members of a group ≤9). This analysis clustered four *spa*

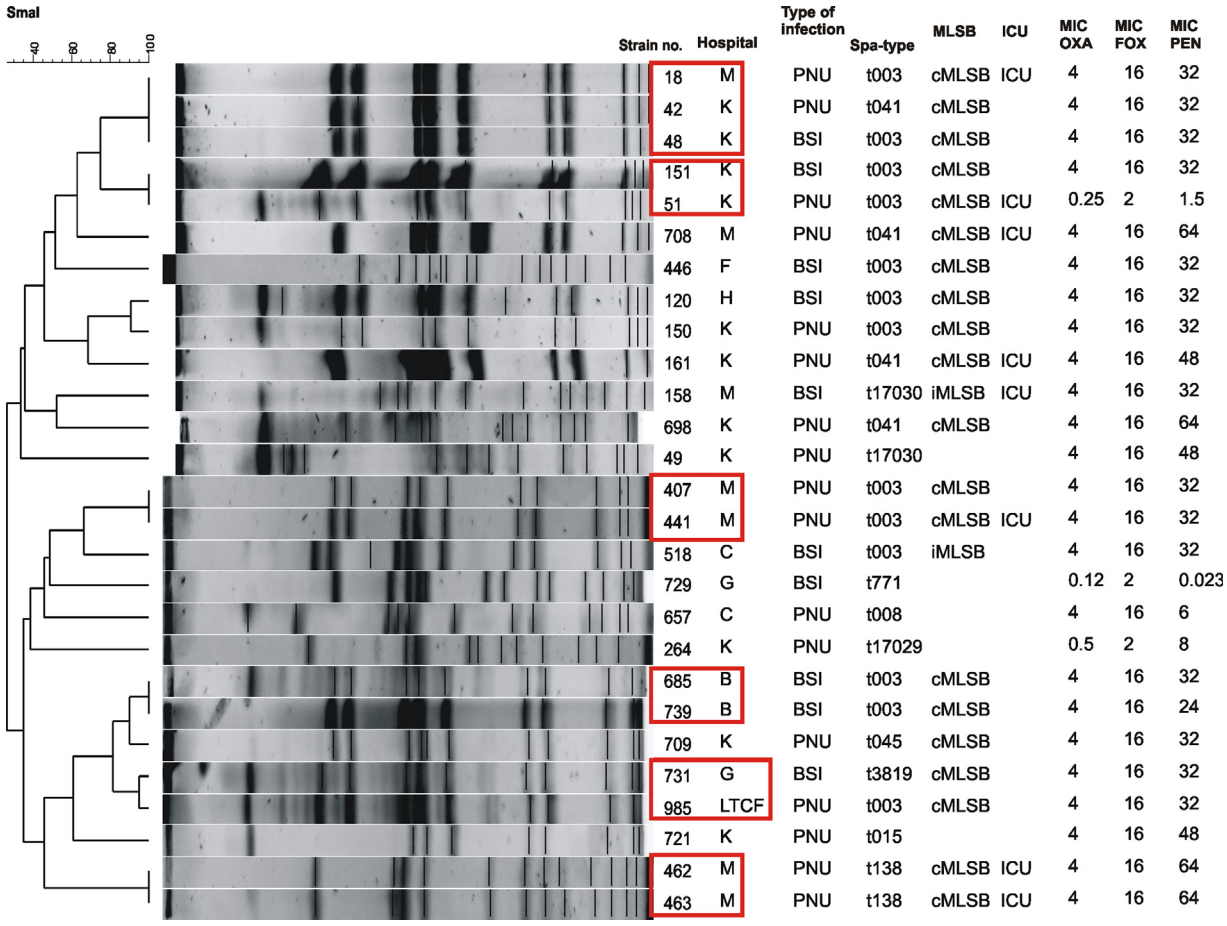

Fig. 1. Pulsed-field gel electrophoresis (PFGE) dendrogram of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates together with *spa* types. Hospitals are designated by letter. PNU, pneumonia; BSI, bloodstream infection; $cMLS_B$, constitutive macrolide–lincosamide–streptogramin B mechanism; $iMLS_B$, inducible macrolide–lincosamide–streptogramin B mechanism; ICU, intensive care unit; MIC, minimum inhibitory concentration; OXA, oxacillin; FOX, cefoxitin; PEN, penicillin. Square frames indicate strains with identical pulsotypes.

Table 3
Results of *spa* typing of methicillin-resistant *Staphylococcus aureus* isolates (*N* = 27).

spa type (no. of isolates)	*spa* repeats	*spa*-CC
t003 (13)	26-17-20-17-12-17-17-16	5
t3819 (1)	26-17- 12-12-17-17-16	5
t045 (1)	26-17-20-17-12-17-16	5
t041 (4)	26-30-17-34-17-20-17-34-17-20-17-12-17-16	5
t015 (1)	08-16-02-16-34-13-17-34-16-34	45
t138 (2)	08-16-02-25-17-24	359
t17029 (1)	04-21-12-20-17-12-12-17-17	Sporadic
t771 (1)	07-23-21-24-33-22-22-17	160
t17030 (2)	07-23-13-23-31-29-17-31-29-17-25-17-25-16-25-28	Sporadic
t008 (1)	11-19-12-21-17-34-24-34-22-25	8

CC, clonal complex.

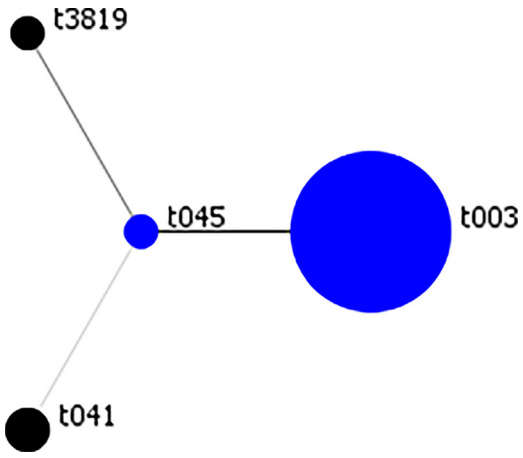

Fig. 2. Analysis of *spa* types clustered by BURP algorithm of the Ridom StaphType software. The predicted founder of a cluster is shown in blue, whilst the other *spa* types are shown in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

types, namely t003, t045, t041 and t3819, represented by 19 isolates, into a single cluster (Fig. 2). The remaining eight isolates represented eight singletons of unrelated *spa* types corresponding to different *S. aureus* cluster types (Table 3). Three isolates have been recognised by BURP as a sporadic clone where based on their *spa* type no CC type could be assigned.

4. Discussion

In earlier reports from Poland the prevalence of MRSA in invasive infections caused by *S. aureus* was within the average values observed in Europe (16% of all *S. aureus* strains in 2013 in Poland, with higher values noted in Mediterranean countries and lower values in the north of the continent) (http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx; accessed 17 December 2015). More importantly, according to ECDC data, the prevalence of MRSA in Poland has been slowly decreasing in recent years, from 23% in 2002 to 15.8% in 2015 [4]. However, the epidemiology of MRSA in non-EU countries is different; the prevalence of MRSA in the USA is significantly higher, with up to 50.7% in BSIs and 42.4% in pneumonia [11]. The prevalence of MRSA is 35% in Belarus and 42% in the Balkans (http://www.euro.who.int/__data/assets/pdf_file/0006/285405/CAESAR-Surveillance-Antimicrobial-Resistance2014.pdf?ua=1; accessed 17 December 2015). Despite the decrease in MRSA invasive infections shown for Poland, the data in the current study obtained from one region in Southern Poland indicate a high risk of MRSA, especially in pneumonia, on a local level. This is also confirmed by a report of Park et al., who also found a greater level of MRSA in pneumonia [12].

The problem of the high proportion of MRSA in pneumonia can be a serious therapeutic problem, especially in the ICU where antibiotic resistance was higher than in other wards.

The low percentages of different virulence factors among studied strains is in accordance with reports by other authors: the difference occurs in the presence of the *lukE* gene, which was present in all of the MRSA strains isolated in Brazil and the USA [13], South Korea [12] and Russia [14]. It indicates a higher risk of infections, including MRSA infections, in hospitalised patients, in whom even strains without significant virulence factors can lead to the development of infection.

Genetic typing of MRSA isolates by PFGE showed relatively high diversity of isolates belonging to the same clonal complex. Only in hospitals with clinical departments (M and K) were closely related isolates detected, suggesting possible hospital transmission events. Non-teaching hospitals were analysed in our previous study, where mostly community-acquired strains had been reported with a large variety among clones [10]. As reported previously, also in this study *spa* typing confirmed the dominant clone CC5 characterised by *spa* type t003 to be the most predominant among hospitals in Southern Poland [10,15]. The high diversity of identified CC5-MRSA isolates obtained from BSI and pneumonia cases in hospitals in Southern Poland suggests their community origin rather the hospital. To further decrease the prevalence of MRSA in Poland it is necessary to implement routine diagnostics to monitor hospitals and to screen patients at admission for MRSA carriage. A search-and-destroy policy for crucial pathogens such as MRSA is necessary to avoid the development of MRSA infection during patient treatment as well as the spread of this pathogen in the hospital setting. It is also important to mention that different regions in Poland may have different MRSA prevalences, which needs to be further investigated.

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Competing interests

None declared.

Ethical approval

This work was approved by the Bioethics Committee of Jagiellonian University Medical College (Krakow, Poland) [no. KBET/227/B/2012].

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