

***Staphylococcus* sp. Involvement in Community Acquired Urinary Tract Infections**

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Abstract

The aim of this study was to investigate the contribution of *Staphylococcus* species to the etiology of community acquired urinary tract infections (UTI) in Romanian population, and to investigate their antibiotic resistance profiles. 6,250 urine samples were collected between August - October 2014 by Synevo Laboratory (Bucharest), out of which the overall isolation rate of *Staphylococcus* spp. was 2.68% (n = 50). *S. saprophyticus* was the most frequent isolated species, being susceptible to methicillin but 28% exhibited an efflux-mediated macrolides resistance phenotype. *S. aureus* was the second most commonly isolated, exhibiting combined MRSA (methicillin-resistant *Staphylococcus aureus*) (encoded by *SSCmec* type III and IVa gene cassettes) and inducible resistance to macrolide, lincosamide, streptogramin B (MLS_{Bi}) phenotypes. The *S. haemolyticus* and *S. lugdunensis* strains were isolated with a lower frequency (2 strains each) and one *S. lugdunensis* strain harboured *SSCmec* type III cassette. Our results highlight differences in the etiology of community acquired UTI from those reported in other geographical areas, proving the necessity of knowing the local etiology and resistance markers in order to design appropriate empirical therapy regimens and to monitor the antimicrobial resistance emergence and spread in community.

Key words: *Staphylococcus* sp., urinary tract infection, MRSA, MLS_{Bi}

1. Introduction

Currently, the antibiotic treatment of UTI is a challenge due to the increasing level of antimicrobial resistance in uropathogenic strains. In order to control the emergence and dissemination of antibiotic resistance is important to determine the etiology and resistance markers in community acquired UTI. Besides *Escherichia coli* and other *Enterobacteriaceae* that are usually responsible for UTI's in community, there are only few literature studies concerning the incidence and the resistance profiles of the staphylococcal species implicated in community-acquired UTI (NETO & al [1], GONZÁLEZ-DOMÍNGUEZ & al [2]). Usually, *S. saprophyticus* is the most common coagulase negative staphylococci causative agent in the immunocompetent host (RAZ & al [3], BECKER & al [4]). Therefore, the objective of this study was to investigate the incidence and resistance profiles of *Staphylococcus* spp. strains isolated from ambulatory urinary tract infections in a Romanian population from the SE region of the country.

2. Materials and methods

Study population

The urine samples collected between August 2014 and October 2014 were referred to a private laboratory performing clinical microbiology services for outpatient clinics and general practitioners – Synevo, Bucharest. This study included 6,250 urine specimens, all collected on UriSWAB (*Copan*, Brescia, Italy).

Strains identification

The streaking pattern was performed with the automated system Walk Away Specimen Processor (WASP- *Copan*, Brescia, Italy) as described (BOURBEAU & al [5]). All specimens with 10^5 and more bacteria/ ml of urine in pure culture were considered positive. All isolates were identified by mass spectrometry using MALDI Biotyper (*Bruker*, Billerica, MA, USA).

Phenotypic analysis of antibiotic susceptibility profiles

Antimicrobial susceptibility testing was performed using Pos MIC Panel Type 32 (MICROSCAN, *Beckman Coulter*, Nyon, Switzerland) and the “D-zone test” for inducible resistance to clindamycin.

Molecular analysis of MRS strains

Each bacterial strain was treated with lysosime (30 mg/ml in TEG buffer - TRIS 0.5M, EDTA 0.5M, Glucose 1M) for 30 min at 37°C prior to PCR analysis. The screening and identification of SCC*mec* cassettes I, II, III, IV, V was performed by multiplex PCR (OLIVEIRA & de LENCASTRE [6], MIHEIRICO & al [7]), and the screening of macrolide resistance genes by simplex PCR as described (DURAN & al [8]).

3. Results and discussions

This is the first study which evaluates the species distribution and resistance features of *Staphylococcus* spp. isolates in the community urinary tract infections in Romanian population. Of the total of 6,250 urine samples, 29.7% ($n = 1867$) were positive, out of which 2.68% ($n = 50$) revealed a *Staphylococcus* spp. infection. Of the total 50 patients with urine cultures positive for *Staphylococcus* spp., 46 were females (92%) and 4 male (8%), with an average age of 30 years. Out of the 50 positive samples, 64% ($n = 32$, 1.71% of all uropathogens) were *S. saprophyticus*, 28% ($n = 14$, 0.75%) *S. aureus*, 4% ($n = 2$, 0.1%), *S. lugdunensis* and 4% ($n = 2$, 0.1%) *S. haemolyticus*. The antibiotic susceptibility testing revealed that the strains were highly susceptible to several tested antibiotic classes, except beta-lactams and macrolides: vancomycin (100%), nitrofurantoin (100%), trimethoprim-sulfamethoxazole (100%), quinolones (98%), aminoglycosides (92%). Six of the 32 *S. saprophyticus* strains (19%) were resistant to penicillin, as compared to *S. aureus* strains, for which 10 of the 14 (71%) strains were penicillin resistant. 11 of 14 *S. aureus* strains (79%) were MRSA. Therefore penicillin could still be considered as a viable therapeutic option in certain staphylococcal infections. The macrolide resistance phenotype (M) was exhibited in 9 of the 32 (28%) *S. saprophyticus* strains (erythromycin resistant, clindamycin susceptible), while in 9 MRSA strains (64%), the i MLSB phenotype was present. One *S. aureus* strain isolated from an old male (75 years old) was resistant to all tested antibiotics, except vancomycin, nitrofurantoin and sulfamethoxazole, exhibiting a multi-drug resistance phenotype. One *S. lugdunensis* was resistant both to penicillin and oxacillin. The isolation rate of *S. saprophyticus* among the isolated staphylococcal strains was lower than that reported in other studies (NEUZILLET & al [9], MARZOUK & al [10], LO & al [11]). *S.*

saprophyticus is a well-known cause of uncomplicated urinary tract infections, especially in young and sexually active women, being after *Escherichia coli*, the second among the most frequent causative agents of acute UTI (CHOI & al [12], HOFMANS & al [13]). The molecular analysis revealed the presence of Type IVa and Type III *SSCmec* gene cassettes in six and, respectively, two *S. aureus* isolates. The genetic support of MLSB phenotype was elucidated in one *S. aureus* strain carrying *ermA* gene. In our study, *S. aureus* was the second most frequent *Staphylococcus* species involved in the community acquired UTI, with an incidence similar to that reported in other studies (SHARMA & al [14]). Even though *S. aureus* has been associated mainly to hospitalized patients, it is more and more frequent in the acute community acquired UTI and the percentage of community-acquired MRSA is also increasing (UDO [15]). As revealed by the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2013 Romania was the country with the highest rate of MRSA isolation (64,5%) (ECDC [16]). *S.lugdunensis* and *S. haemolyticus* were isolated with a lower frequency, compared to *S. saprophyticus* and *S. aureus*. However, although *S. lugdunensis* is reported especially in hospital infections, particularly involving soft tissues and bacteremia, it should never be dismissed as a contaminant without careful review (KLOTCHKO & al [17]). The percentage of *S. haemolyticus* was much lower than that reported by other studies (KLEEMAN & al [18], OZTÜRKERİ & al [19]). *SSCmec* Type IVa cassette was also reported by other studies in community-onset methicillin-resistant *S. aureus* isolated in Spain, Hokkaido, the main northern island of Japan, Taiwan, Vladivostock, Russia (GONZÁLEZ-DOMÍNGUEZ & al [2], KAWAGUCHIYA & al [20], HUANG & CHEN [21], BARANOVICH & al [22]). While *SSCmec* IV are usually related to community acquired MRSA infections (ITO & al [23]), *SSCmec* III elements are usually associated with hospital-acquired MRSA (CHAMBERS & al [24]).

4. Conclusions

This is the first study focused on the isolation rate and resistance profiles of *Staphylococcus* spp. isolated from community acquired urinary tract infections in Romania. The study was conducted on significant number of samples analyzed in 2014 and revealed an overall percentage of 2.68% staphylococcal species in the positive urine cultures. The most frequent causative species was *S. saprophyticus*, followed by *S. aureus*, *S. lugdunensis* and *S. haemolyticus*. The result regarding the isolation rate and the antibiotic susceptibility profiles were different from those reported by other studies, therefore, knowing the incidence and resistance markers of community acquired UTI in our geographical area is crucial for the design of empirical therapy regimens and for the monitoring of antimicrobial resistance emergence and spread in community.

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