Correlation of phenotypic tests with the presence of the blaZ gene for detection of beta-lactamase

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ABSTRACT

Staphylococcus aureus and Staphylococcus saprophyticus are the most common and most important staphylococcal species associated with urinary tract infections. The objective of the present study was to compare and to evaluate the accuracy of four phenotypic methods for the detection of beta-lactamase production in Staphylococcus spp. Seventy-three strains produced a halo with a diameter \( \leq 28 \text{ mm} \) (penicillin resistant) and all of them were positive for the blaZ gene. Among the 28 susceptible strain (halo \( >29 \text{ mm} \)), 23 carried the blaZ gene and five did not. The zone edge test was the most sensitive (90.3%), followed by MIC determination (85.5%), but the specificity of the former was low (40.0%). The nitrocefin test was the least sensitive (28.9%). However, the nitrocefin test together with the disk diffusion method showed the highest specificity (100%). The present results demonstrated that the zone edge test was the most sensitive phenotypic test for detection of beta-lactamase, although it is still not an ideal test to detect this type of resistance since its specificity was low. However, the inhibition halo diameter of the penicillin disk can be used together with the zone edge test since the same disk is employed in the two tests. Combined analysis of the two tests shows a sensitivity of 90.3% and specificity of 100%, proving better sensitivity, especially for S. saprophyticus. This is a low-cost test of easy application and interpretation that can be used in small and medium-sized laboratories where susceptibility testing is usually performed by the disk diffusion method.

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Introduction

The most common etiological agents involved in urinary tract infections (UTIs) include enterobacteria, non-fermenting Gram-negative bacilli, fungi, enterococci, and staphylococci. *Staphylococcus aureus* and *Staphylococcus saprophyticus* are the most common and most important staphylococcal species associated with UTIs; however, other coagulase-negative staphylococci have gained importance in recent years.

*S. saprophyticus* is the second most common causative agent of acute UTI in the community after *Escherichia coli* and is isolated mainly from urine of sexually active young women,\(^1\)\(^2\) causing symptoms that are undistinguishable from those induced by *E. coli*. Cases of sepsis and pyelonephritis caused by this microorganism have also been reported.\(^3\)\(^4\)

The occurrence of penicillin resistance in *S. aureus* isolates recovered from hospitalized patients has been reported shortly after the introduction of penicillin in 1941.\(^5\)\(^6\) At the beginning of the 1970s, resistance to penicillin became common among nosocomial *S. aureus* isolates (85–90%) and shortly thereafter penicillinase-producing strains rapidly spread in the community.\(^7\)\(^8\) Today, more than 90% of *S. aureus* strains isolated from humans are resistant to penicillin.\(^10\)–\(^12\)

Penicillin resistance in *staphylococci* is mediated by the production of a penicillinase encoded by the *blaZ* gene, which inactivates penicillin by hydrolysis of the beta-lactam ring.\(^13\)\(^14\) Four types of penicillinases (A–D) have been described in *S. aureus*, all of them belonging to Ambler class A beta-lactamases.\(^14\)\(^15\) Penicillinases type A, C and D are usually plasmid encoded, while type B is located on the chromosome.\(^16\)\(^17\)

Penicillin-susceptible *staphylococci* are susceptible to all beta-lactam antibiotics. Penicillin-resistant, oxacillin-susceptible strains are resistant to penicillin-labile penicillins, but are susceptible to other penicillin-labile penicillins, beta-lactam/beta-lactamase inhibitor combinations, cephalosporins, and carbapenems. Oxacillin-resistant *staphylococci* are resistant to all currently available beta-lactam antibiotics, except for new cephalosporins with anti-MRSA activity (cef-taroline and cefotibiprole).

At present, most *staphylococci*, either coagulase positive or coagulase negative, are resistant to penicillin G, as well as to penicillin V, ampicillin, amoxicillin, and carbenicillin.\(^18\)\(^19\) Penicillin susceptibility testing of *staphylococcal* isolates is performed in most laboratories by the agar disk diffusion. The Clinical and Laboratory Standards Institute\(^20\) defines penicillin susceptibility as a minimum inhibitory concentration (MIC) of penicillin ≤0.12 μg/mL or as the formation of an inhibition halo ≥29 mm by the disk diffusion method (Kirby-Bauer), in combination with a negative result in the chromogenic cephalosporin assay (nitrocefin disk) for detection of beta-lactamase production. Although the disk diffusion and nitrocefin tests are accepted as methods for determining penicillin susceptibility, studies suggest that these methods do not reliably detect beta-lactamase production.\(^21\)\(^22\)

Other methods that have been proposed to improve the sensitivity of beta-lactamase detection in *staphylococci* include the clover-leaf test\(^23\)\(^24\) and observation of the appearance of the inhibition zone edge around penicillin G disks (zone edge test). However, little information about the reliability of the latter phenomenon is available.\(^23\)\(^27\) especially for coagulase-negative *staphylococci*.

In 2012, the CLSI\(^28\) started to recommend the zone edge test. This test was found to be more sensitive than the nitrocefin method in detecting beta-lactamase production in *S. aureus* when only one test was performed. However, laboratories could choose to perform first the nitrocefin test and, if the test were positive, to report the isolate to be beta-lactamase positive or penicillin resistant. If the nitrocefin test were negative, the zone edge test should be performed before reporting an isolate to be penicillin susceptible.

In view of the high prevalence of penicillin resistance among *staphylococci*, penicillin is rarely used as antistaphylococcal treatment mainly UTIs, where there are several other antimicrobial agents that may be used. However, little information about these tests is available for coagulase-negative *staphylococci* and better assessment of the detection of beta-lactamase production in these species is required. The objective of the present study was to compare and to evaluate the accuracy of the following four phenotypic methods for the detection of beta-lactamase production in *Staphylococcus* spp.: appearance of the inhibition zone edge around the penicillin G disk, disk diffusion, MIC determination, and nitrocefin disk test. Detection of the *blaZ* gene by the polymerase chain reaction (PCR) was used as a reference.

Materials and methods

Strains

One hundred one *Staphylococcus* spp. strains isolated from urine samples of patients seen in wards, outpatient clinics, the emergency department and several health centers of Botucatu and region were used in this study. The isolates were sent to the Laboratory of Microbiology of the University Hospital (HC), Botucatu Medical School (FMB), between March 10 and November 14, 2008. The project was approved by the Ethics Committee of FMB, UNESP (OF.416/08-CEP).

Inclusion and exclusion criteria

Patients of both genders and all ages, whose urine cultures were positive for *Staphylococcus* spp. and considered compatible with UTI, with a colony-forming unit count (CFU) ≥10⁵ per mL urine according to the criteria of Kass,\(^29\) were included in the study.

Strains isolated from urine catheters, suprapubic punctures and positive urine cultures containing <10³ CFU/mL were excluded.

Sample size calculation

The sample size was calculated using the formula of Fisher and Belle,\(^30\) adopting a 95% confidence interval and precision of 5% for the expected prevalence of patients with UTI. The proportion of patients with UTI caused by *Staphylococcus* spp., which was 5% in a study conducted at the Laboratory of Microbiology of HC-FMB, was used as a basis.
Although the sample size calculation indicated 73 isolates as the minimum number, all Staphylococcus spp. strains isolated during the study period, which met the inclusion and exclusion criteria, were used. A total of 101 isolates were thus included.

**Nucleic acid extraction**

Total nucleic acid was extracted from Staphylococcus spp. strains cultured on blood agar, individually inoculated into BHI broth, and incubated at 37 °C for 24 h. The Illustra Kit (GE Healthcare, Chalfont, England) was used for extraction according to manufacturer recommendations and the extracted DNA was stored under refrigeration at −20 °C.

**Genotypic identification of Staphylococcus spp.**

The Staphylococcus spp. isolates were submitted to genotypic identification using primers targeting conserved sequences adjacent to the 16S and 23S genes. This method described by Barry et al. and Couto et al. is known as internal transcribed spacer-polymerase chain reaction (ITS-PCR). The technique was carried out as described by Couto et al., using primers G1 (5'-GAA GTC GTA ACA AGG-3') and L1 (5'-CAA GGC ATC CAC CGT-3').

**Antimicrobial susceptibility testing by the disk diffusion method**

The susceptibility profile to penicillin G 10 UN (Oxoid, Basingstoke, England) was evaluated using the criteria recommended by the CLSI. For inoculum preparation, a direct suspension of colonies (pure 24-h culture) in 0.9% saline (Fresenius Kabi, Aquiraz, Brazil), corresponding to a 0.5 McFarland turbidity standard, was used. After homogenization of the suspension, a sterile swab was pressed against the inner wall of the tube for removal of excess inoculum and seeded in four different directions onto the surface of Mueller-Hinton agar plates (Oxoid, Basingstoke, England). The plates were left to stand for 5–15 min at room temperature to allow complete absorption of the inoculum by the agar before application of the disks. The plates were incubated for 24 h at 35 °C and the penicillin inhibition halos were measured after this period by illuminating the surface with reflected light.

**Determination of the minimum inhibitory concentration of antimicrobials**

The MIC against the S. aureus and coagulase-negative staphylococcal isolates was determined using E-test® strips (AB Biodisk, Solna, Sweden) for penicillin G at an MIC interval of 0.002–32.0 μg/mL. The procedures of inoculum preparation, plating and incubation were the same as those described for the disk diffusion method. The MIC was determined at the point of intersection between the strip’s scale and the ellipse of the zone of bacterial growth inhibition. Values ≤0.12 μg/mL and >0.25 μg/mL were defined as susceptible and resistant, respectively. Strains exhibiting MICs >0.12 μg/mL and <0.25 μg/mL were considered resistant.

**Nitrocefin disk test for detection of beta-lactamase production**

Production of beta-lactamase was detected using disks impregnated with nitrocefin (chromogenic cephalosporin) (Becton Dickinson, Sparks, USA). The test is based on the release of a chromogenic radical, which causes a color change when the beta-lactam ring is broken open by the action of beta-lactamase.

The disk was moistened with one or two drops of sterile distilled water and deposited near an oxacillin E-test® strip on a culture previously incubated for 24 h at 35 °C on a Mueller-Hinton agar plate. The disks were analyzed after 5 min. A positive reaction was defined by the development of a red color and a negative reaction by the lack of color change. In the case of beta-lactamase-negative strains, the reaction was reexamined after 1 h according to manufacturer recommendations. Positive (S. aureus ATCC 35913) and negative (Staphylococcus xylosus ATCC 29979) control strains were included.

**Zone edge test for detection of beta-lactamase production**

The production of beta-lactamase was evaluated based on the appearance of the inhibition zone edge around the penicillin G disk (Oxoid, Basingstoke, England) obtained by the disk diffusion method. The test was defined as negative when the appearance of the edge was fuzzy like a “beach” and as positive when the edge was sharp like a “cliff.”

**Detection of the blaZ gene by PCR**

The PCR mixtures contained, in a total volume of 50 μL, 200 μM of each dNTP, 1 μM of each primer (stau-blaZ-forward: 5'-AAAAGATGATAAGTTGCTTATTCC-3' and stau-blaZ-reverse: 5'-TGGCTGGACCCATTCCAAGC-3'), 10 μL DNA, 1.25 U Taq polymerase (GE Healthcare, München, Germany), and reaction buffer provided by the manufacturer. Amplification was carried out in a PTC-100™ thermocycler (MJ Research, Watertown, USA) using the parameters described by Kaase et al.: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 7 min.

The reaction was positive when a 421-bp DNA fragment was observed. blaZ-positive (S. aureus ATCC 29213) and negative (S. aureus ATCC 25923) reference strains were included in all reactions.

**Results**

Among the 101 isolates analyzed by ITS-PCR, 57 were identified as S. saprophyticus, 17 as S. aureus, 16 as Staphylococcus epidermidis, eight as Staphylococcus haemolyticus, two as Staphylococcus warneri, and one as Staphylococcus lugdunensis.

Twenty-eight (27.7%) isolates were susceptible to penicillin by the disk diffusion method and 16 (15.8%) by the E-test®
Among the 28 isolates susceptible to penicillin by the disk diffusion method, 22 (78.6%) were *S. saprophyticus*, three (10.7%) were *S. aureus*, two (7.1%) were *S. epidermidis*, and one (3.6%) was *S. lugdunensis*.

The nitrocefin method detected beta-lactamase production in only one (4.5%) *S. saprophyticus* isolate among the 28 isolates susceptible to penicillin by the disk diffusion method; however, the zone edge test was positive in 18 (81.8%) (Fig. 2). Fifteen (68.2%) *S. saprophyticus* isolates were resistant to penicillin by the E-test® and 19 carried the blaZ gene. Three isolates *S. aureus* and two *S. epidermidis* were susceptible to penicillin by the two methods (disk diffusion and E-test®) and tested negative in the nitrocefin and zone edge tests (Fig. 3). However, three isolates carried the blaZ gene (two *S. aureus* and one *S. epidermidis*). The *S. lugdunensis* was resistant to penicillin only by E-test® and blaZ gene positive although tested negative in the nitrocefin and zone edge tests (Table 1).

Seventy-three isolates exhibited a halo diameter ≤28 mm (resistant) for penicillin and all of them were positive for the blaZ gene (Fig. 4). Among the 28 susceptible isolates (halo ≥29 mm), 23 carried the blaZ gene and five did not (Fig. 5).

As can be seen in Table 2, the zone edge test was the most sensitive (90.3%), followed by MIC determination (85.5%). In contrast, the zone edge test showed low specificity (40%) and the nitrocefin test was the least sensitive (28.9%). The highest specificity (100%) was observed for the nitrocefin test in combination with the disk diffusion method.

(Fig. 1). The 73 remaining isolates (72.3%) were resistant to penicillin by the disk diffusion method, with an inhibition halo ≤28 mm and MIC ≥0.12 μg/mL, except for four isolates that exhibited MIC <0.12 μg/mL. The zone edge test identified three false-positive isolates and eight false-negative isolates among the 88 strains studied. Thirteen isolates could not be evaluated due to the lack of formation of an inhibition halo.

![Fig. 1 – Resistance to penicillin by the disk diffusion method and E-test®.](image)

![Fig. 2 – Positive zone edge test for the detection of beta-lactamase production (sharp edge).](image)

![Fig. 3 – Negative zone edge test for the detection of beta-lactamase production (fuzzy edge).](image)
Table 1 – Detection of beta-lactamase production by phenotypic and genotypic methods in isolates that were susceptible to penicillin by the disk diffusion method.

| Species            | Penicillin Disk (mm) | Penicillin MIC (μg/mL) | Detection of beta-lactamase production |
|--------------------|----------------------|------------------------|----------------------------------------|
|                    | ≥29a                  | ≤0.12b                 | ≥0.25c                                 |
| S. saprophyticus    | 22 (78.6)             | 7 (31.8)               | Positive                               |
| S. aureus          | 3 (10.7)              | 3 (100)                | Positive                               |
| S. epidermidis      | 2 (7.1)               | 2 (100)                | Positive                               |
| S. lugdunensis     | 1 (3.6)               | 0 (0)                  | Positive                               |
| Total              | 28 (100)              | 12 (42.9)              | 16 (57.1)                              |

MIC, minimum inhibitory concentration.

* Halo diameter ≥29 mm = susceptible.

* MIC ≤0.12 μg/mL = susceptible.

* MIC ≥0.25 μg/mL = resistant.

Fig. 4 – Agarose gel electrophoresis for detection of the blaZ gene (421 bp) by PCR. Lanes 4–8 and 13–22: positive isolates; line 9 is positive an 13 is negative; negative isolates; 3: negative control (S. aureus ATCC 25923); 2: positive control (S. aureus ATCC 29213); 23: water; 1: molecular weight marker (100 bp).

Discussion

Although UTIs caused by S. saprophyticus have been documented, the antimicrobial resistance and dissemination of this species are not well studied. Today, most of the isolated staphylococcal species, either coagulase positive or coagulase negative, are resistant to penicillin. In view of the high prevalence of penicillin resistance in staphylococci, this antibiotic is rarely used as antistaphylococcal treatment. However, prolonged treatment with penicillin may be desirable in certain circumstances, as long as the susceptibility test result is reliable.

The best phenotypic method to detect penicillin resistance in Staphylococcus spp. continues to be a challenge. In this respect, the CLSI is evaluating recommendations for the detection of this type of resistance. Several methods have been proposed to improve the sensitivity of beta-lactamase detection in staphylococci, including the clover-leaf test, nitrocefin disk method, and zone edge test. However, little information

Table 2 – Sensitivity and specificity of the methods used for the detection of beta-lactamase in Staphylococcus spp.

| Accuracy test | Disk diffusion | MIC | Nitrocefin | Zone edge test |
|---------------|----------------|-----|------------|---------------|
| Sensitivity (%) | 72.2           | 85.5 | 28.9       | 90.3          |
| Specificity (%) | 100.0          | 80.0 | 100.0      | 40.0          |

MIC, minimum inhibitory concentration.

Fig. 5 – Correlation between halo size and presence of the blaZ gene.
is available regarding the reliability of these tests, especially in coagulase-negative staphylococci.

In the present study, only 28 (27.7%) of the 101 isolates analyzed were susceptible to penicillin by the disk diffusion method and 12 (11.8%) by the E-test®. The remaining 73 (72.3%) penicillin-resistant isolates exhibited an inhibition halo ≤28 mm and MIC ≥0.12 µg/mL, demonstrating low susceptibility to this antimicrobial agent. This susceptibility is even lower when considering that 19 of the 28 susceptible isolates in the disk diffusion test carried the blaZ gene, a fact impairing any attempt of treatment of UTIs with this antimicrobial drug, especially empirical therapy.

Kaase et al. studied 197 S. aureus isolates with MIC ≤0.12 µg/mL. Twenty-eight (14.2%) of these isolates were positive for the blaZ gene, including 20 (71.4%) detected by the zone edge test and 11 (39.3%) by the nitrocefin test. In the present study, 12 (11.8%) of the 28 isolates susceptible to penicillin by the disk diffusion method had MIC ≤0.12 µg/mL. However, 18 (64.3%) tested positive in the zone edge test and only one (3.6%) in the nitrocefin method. These values are similar to those reported by Kaase et al. for the zone edge test, but are lower than those observed by these authors for the nitrocefin method.

The breakpoint recommended by the CLSI to classify penicillin-resistant isolates by the disk diffusion method showed a sensitivity of 72.2%, a value higher than the 57.1% reported by Kaase et al. In this study, the sensitivity of the nitrocefin test (28.9%) was lower than that obtained by these authors (39.3%) and by others: 82.0%, 33 86.2%, 34 95.6%, 23 70.8–97.9% depending on the induction method and agar used, 24 and 62.1–100% depending on the manufacturer. 26 The large difference in the sensitivity of the nitrocefin test between the present study and the studies cited may be related to the fact that we mainly evaluated S. saprophyticus.

McDougal and Thornberry evaluated the production of beta-lactamase in 66 S. aureus isolates using the nitrocefin disk method and all strains tested positive. However, the change in color was weak when the penicillin MIC was less than ≤16 µg/mL. In the present study, all S. saprophyticus isolates with a negative nitrocefin disk test exhibited penicillin MICs of 0.125–0.38 µg/mL, except for one isolate with an MIC of 0.94 µg/mL, while three of the two positive isolates had higher MICs (1.0 and 1.5 µg/mL) and one isolated had an MIC of 0.19 µg/mL.

More recently, Pitkälä et al. 26 used detection of the blaZ gene by PCR as a reference method to evaluate different commercial tests for detection of beta-lactamase production in Staphylococcus spp., including the nitrocefin disk method. The authors found 19 isolates with a very weak reaction to nitrocefin; of these, 15 were positive for the blaZ gene. In this present study, only one of the 22 S. saprophyticus isolates tested positive in the nitrocefin test, but 19 were positive for the blaZ gene. These results agree with the findings of Hovelius and Mardel who showed that the chromogenic cephalosporin (nitrocefin) does not detect beta-lactamase production in S. saprophyticus, a fact explaining the low sensitivity of the nitrocefin disk in detecting beta-lactamase production observed here, especially in S. saprophyticus. In contrast, Haveri et al. 22 found high agreement between the nitrocefin test and the presence of the blaZ gene in S. aureus. Although the nitrocefin disk method is a commonly used test for beta-lactamase detection, the enzymatic reactions may be incomplete, leading to a large number of false-negative results. This fact may explain the large number of beta-lactamase-negative S. saprophyticus isolates.

Twenty-three (82.1%) of the 28 isolates that were susceptible to penicillin by the disk diffusion method were blaZ-positive. This rate is much higher than those reported by Kaase et al. 15 and El Feghaly et al. 37 who evaluated 105 penicillin-susceptible S. aureus isolates. In those studies, 14.2% and 9.5% of the S. aureus isolates phenotypically susceptible to penicillin carried the blaZ gene, respectively. This marked difference is likely due to the large number of S. saprophyticus isolates tested in the present study.

All isolates with a halo diameter ≤28 mm carried the blaZ gene. However, a large inhibition halo did not rule out the presence of the blaZ gene, as also reported by Kaase et al. 15 and El Feghaly et al. 37 In the study of El Feghaly et al., 37 blaZ-positive isolates by the disk diffusion method exhibited an average halo size of 34 mm, while the inhibition halo of blaZ-negative isolates was greater than 38 mm. However, we found three isolates with an inhibition halo >40 mm (one S. aureus, one S. epidermidis, and one S. lugdunensis), which were positive for the blaZ gene. These findings agree with Kaase et al. 15 and El Feghaly et al. 37 who suggested conventional (phenotypic) methods to be unable to adequately detect penicillin resistance in staphylococcal isolates.

El Feghaly et al. 37 also suggested that changing the breakpoint of the penicillin inhibition halo from 29 to 35 mm would improve sensitivity of the test. A similar observation was made by Latham et al. for S. saprophyticus. These authors found that isolates with a diameter <31 mm were all beta-lactamase positive. The use of this new breakpoint would permit to detect penicillin resistance in all S. saprophyticus isolates of the present study, which exhibited inhibition halos ranging in size from 29 to 35 mm. However, three blaZ-negative isolates would be considered positive if this new criterion was used.

With respect to the MIC of penicillin, Kaase et al. 15 found that all isolates with an MIC of 0.03 µg/mL determined with the Vitek 2 system were blaZ-negative, while five (6.2%) of 81 isolates with an MIC of 0.06 µg/mL and 23 (23.2%) of 99 isolates with an MIC of 0.12 µg/mL were blaZ-positive. Among the blaZ-negative isolates, two S. saprophyticus strains exhibited an MIC of 0.125 µg/mL and one an MIC of 0.19 µg/mL, one S. aureus had an MIC of 0.032 µg/mL and one S. epidermidis had an MIC of 0.012 µg/mL. The MIC of the other blaZ-positive isolates ranged from 0.012 to 0.94 µg/mL, demonstrating that determination of the MIC also does not reliably detect this type of resistance. This difficulty has also been reported by Haveri et al. 22 who found five blaZ-negative isolates with MIC ≥0.25 µg/mL and 26/211 (12.3%) susceptible isolates with MIC of 0.06–0.125 µg/mL that carried the blaZ gene.

In the present study, the zone edge test was the most sensitive phenotypic test (90.3%). A similar result has been reported by Kaase et al. 15 who evaluated the sensitivity of five phenotypic methods for detection of beta-lactamase and found a sensitivity of 71.4% for the zone edge test. In contrast, in the study of El Feghaly et al., 37 the most sensitive phenotypic test was the clover-leaf test (60.0%). According to these authors, the zone edge test is highly subjective and its interpretation may vary depending on the examiner. However, the test was
analyzed by two examiners in the present study and there was no discrepancy between results.

The present results demonstrated that the zone edge test was the most sensitive phenotypic test for detection of beta-lactamase, although it is still not an ideal test to detect this type of resistance since its specificity was low. Determination of the MIC and the disk diffusion method provided better results than the nitrocefin test and the zone edge test. However, the inhibition halo diameter of the penicillin disk can be used together with the zone edge test since the same disk is employed in the two tests. Combined analysis of the two tests shows a sensitivity of 90.3% and specificity of 100%, proving better sensitivity, especially for S. saprophyticus. This is a low-cost test of easy application and interpretation that can be used in small and medium-sized laboratories where susceptibility testing is usually performed by the disk diffusion method.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support (304729/2014-0).

REFERENCES

1. Raz R, Colodner R, Kunin CM. Who are you – Staphylococcus saprophyticus? Clin Infect Dis. 2005;40:896–898.
2. Eriksson A, Giske CG, Ternhag A. The relative importance of Staphylococcus saprophyticus as a urinary tract pathogen: distribution of bacteria among urinary samples analysed during 1 year at a major Swedish laboratory. Acta Pathol Microbiol Immunol Scand. 2013;121(1):72–78.
3. Lee W, Carpenter RJ, Phillips LE, Faro S. Pyelonephritis and sepsis due S. saprophyticus. J Infect Dis. 1987;155(5):1079–1080.
4. Collodge CL. Staphylococcus saprophyticus bacteremia. J Infect Dis. 1989;157(1):215.
5. Kirby WM. Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. Science. 1944;99:452–453.
6. Barber M, Rozwadowska-Dowzenko M. Infection by penicillin-resistant staphylococci. Lancet. 1948;2:641–644.
7. Jessen O, Rosendal K, Bulew P, Faber V, Erikson KR. Changing staphylococci and staphylococcal infections. A ten-year study of bacteria and cases of bacteremia. N Engl J Med. 1969;281:627–635.
8. Ross S, Rodriguez W, Controni G, Khan W. Staphylococcal susceptibility to penicillin G. The changing pattern among community strains. JAMA. 1974;229:1075–1077.
9. Chambers HF. The changing epidemiology of Staphylococcus aureus? Emerg Infect Dis. 2001;7:178–182.
10. Livermore DM. Antibiotic resistance in staphylococci. Int J Antimicrob Agents. 2000;16(1):3–10.
11. Lowy FD. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003;111:1265–1273.
12. Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2009;7:629–641.
13. Zhang HZ, Hackbarth CJ, Chansky KM, Chambers HF. A proteolytic transmembrane signaling pathway and resistance to beta-lactams in staphylococci. Science. 2001;291:1962–1965.
14. Zygmunt DJ, Stratton CW, Kernode DS. Characterization of four beta-lactamas produced by Staphylococcus aureus. Antimicrob Agents Chemother. 1992;36:440–445.
15. Kaae M, Lenga S, Friedrich S, et al. Comparison of phenotypic methods for penicillinase detection in Staphylococcus aureus. Clin Microbiol Infect. 2008;14(6):614–616.
16. Olsen JE, Christensen H, Aurestrup FM. Diversity and evolution of blaZ from Staphylococcus aureus and coagulase-negative staphylococci. J Antimicrob Chemother. 2006;57:450–460.
17. Nannini EC, Stryjewski ME, Singh KV, Bourgogne A, Rude TH, Corey GR. Inoculum effect with cefazolin among clinical isolates of methicillin-susceptible Staphylococcus aureus: frequency and possible cause of cefazolin treatment failure. Antimicrob Agents Chemother. 2009;53:3437–3441.
18. Medeiros AA. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin Infect Dis. 1997;24(1):19–45.
19. Tavares W. Manual de Antibióticos e Quimioterápicos Anti-infecciosos. 9th ed. São Paulo: Athenaeum; 2001.
20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 25th Informational Supplement (M100-S25). Wayne, PA: CLSI; 2015.
21. Gill VJ, Manning CB, Ingalls CM. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. J Clin Microbiol. 1981;14:437–440.
22. Haveri M, Suominen S, Rantalä I, Honkanen-Buzalski T, Pyorala S. Comparison of phenotypic and genotypic detection of penicillin G resistance of Staphylococcus aureus isolated from bovine intramammary infection. Vet Microbiol. 2005;106:97–102.
23. Jarlev JO, Rosdahl VT. Quantitative determination of beta-lactamase production in Staphylococcus aureus strains compared to qualitative testing by a microbiological clover leaf test, a chromogenic cephalosporin test and a iodometric test. Acta Pathol Microbiol Immunol Scand. 1986;89:415–421.
24. Petersson AC, Ellässon I, Kamme C, Miörner H. Evaluation of four qualitative methods for detection of beta-lactamase production in Staphylococcus and Micrococcus species. Eur J Clin Microbiol Infect Dis. 1989;8:962–967.
25. Bergan T, Bruun JN, Digranes A, Lingaas E, Melby KK, Sander J. Susceptibility testing of bacteria and fungi. Report from “the Norwegian Working Group on Antibiotics”. Scand J Infect Dis Suppl. 1997;103:1–36.
26. Pitkälä A, Salminen M, Hietala P, Rautamaa T, Elokkala J. Detection of beta-lactamase-producing staphylococci. J Clin Microbiol. 2007;45:2031–2033.
27. Ingram GI. Formation of clear zones with “sensitive” and “resistant” Staphylococcus aureus in penicillin plate assays. J Gen Microbiol. 1951;5:22–29.
28. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 21st Informational Supplement (M100-S21). Wayne, PA: CLSI, 2012.
29. Kass EH. Asymptomatic infections of the urinary tract. Trans Assoc Am Phys. 1956;69:56–64.
30. Fisher LD, Belle GV. Biostatistics: A Methodology for Health Science. New York: John Wiley; 1993.
31. Barry T, Colleran G, Glennon M, Dunican LK, Gannon F. The 16S/23S ribosomal spacer region as a target for DNA probes to identify eubacteria. PCR Methods Appl. 1991;1:51–56.
32. Couto I, Pereira S, Miragaia M, Sanches IS, Lencastre H. Identification of clinical staphylococcal isolates from humans by internal transcribed spacer PCR. J Clin Microbiol. 2001;3099–3103.

33. Banic S, Dragas AZ, Fiser J. Comparison of the efficiency of three methods for the determination of staphylococcal betalactamase. J Chemother. 1990;2:17–19.

34. Bruun B, Gahrn-Hansen B. Mecillinam susceptibility as an indicator of beta-lactamase production in Staphylococcus aureus. Clin Microbiol Infect. 2002;8:122–124.

35. McDougal LK, Thornsberry C. The role of the β-lactamases in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. J Clin Microbiol. 1986;23:832–839.

36. Hovelius B, Mardh P. Staphylococcus saprophyticus as a common cause of urinary tract infections. Rev Infect Dis. 1984;6(3):328–337.

37. El Feghaly RE, Stamm JE, Fritz SA, Burnham CD. Presence of the blaZ beta-lactamase gene in isolates of Staphylococcus aureus that appear penicillin susceptible by conventional phenotypic methods. Diagn Microbiol Infect Dis. 2012;74:388–393.

38. Latham RH, Zeleznik D, Minshew BH, Schoenknecht FD, Stamm WE. Staphylococcus saprophyticus β-lactamase production and disk diffusion susceptibility testing for three β-lactam antimicrobial agents. Antimicrob Agents Chemother. 1984;26:670–672.