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SHORT COMMUNICATION

Comparison of Three Rapid Tests for the Detection of β-Lactamase Production among Bacterial Isolates

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ABSTRACT

The current study compares the performance and evaluated the practicality of three rapid detection methods for β-lactamase production among 48 veterinary bacterial isolates (16 *S. aureus*, 18 *E. coli*, and 14 *S. typhimurium*) in Korea. Antimicrobial susceptibility was determined by broth microdilution method and β-lactamase production was detected by iodometric, acidimetric, and chromogenic methods. Seventy five percent of *S. aureus*, 44% of *E. coli*, and 35% of *S. typhimurium* were resistant to penicillin, ampicillin, and amoxicillin, respectively. Overall, the iodometric test yielded the highest positive results (91.67%), followed by chromogenic test (66.67%) and acidimetric test (47.92%). The sensitivities and specificities of the three different tests varied for *S. aureus*, *E. coli*, and *S. typhimurium* isolates. Our results showed that these test methods may applicable to monitor β-lactamase production among various microorganisms and to predict β-lactam antibiotic resistance.

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INTRODUCTION

Antibiotic resistance is an increasing public health problem (Haag *et al.*, 2013; Asi *et al.*, 2015; Cengiz *et al*., 2015). β-lactam antibiotics such as penicillin are the most widely used antibiotics for treatment of bacterial infections in both veterinary and human medicine (Samant and Pai, 2012). However, production of β-lactamases is the primary cause of microbial resistance to β-lactams such as penicillins and cephalosporins (Gajul *et al.*, 2012; Livermore and Brown, 2001). The β-lactamases inactivates both penicillins and some cephalosporins by hydrolyzing the beta lactam bond (Nicholas-Chanoine 1996). Plasmid-encoded β-lactamases cleave the β-lactam ring of penicillin and hydrolyze various β-lactams (Li *et al.*, 2007; Samant and Pai, 2012). β-lactamase has been detected by various rapid methods (Gajul *et al.*, 2012; PitKala *et al.*, 2007) including chromogenic, acidimetric, and iodometric methods. The chromogenic test utilizes a chromogenic cephalosporin that changes from light yellow to deep red upon hydrolysis (Shrestha and Rana, 2014). This antibiotic is sensitive to hydrolysis by all known β-lactamases produced by Gram-positive and Gram-negative bacteria (Livermore and Brown, 2001). The acidimetric test is based on hydrolysis of the β-lactam ring in benzylpenicillin, which results in the production of penicillonic acid. This causes acidification of the bacterial suspension and a pH-mediated change in color from purple pink to yellow (Samant and Pai, 2012). The iodometric method detects the color change is caused by the removal of iodine based on a reducing action of the β-lactamase hydrolysis product (Livermore and Brown, 2001). These tests are relatively fast, taking only 2-4 hours to detect β-lactamase activity. The aim of this study was to compare the performance and evaluate the practicality of three rapid test methods for detection of β-lactamase production among veterinary bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*) isolated in Korea.

MATERIALS AND METHODS

All the 48 bacterial isolates were received from the Korean Veterinary Antimicrobial Resistance Monitoring System (KVARMS) in 2014. Among, 16 *S. aureus* strains were from cattle carcasses (4), pig carcasses (7), and

chicken carcasses (5), 18 *E. coli* strains from cattle feces (6), pig feces (6), chicken feces (2), cattle carcasses (2), pig carcasses (2), and14 *S. typhimurium* strains from cattle feces (3), pig feces (3), chicken feces (2), cattle carcasses (2), pig carcasses (2), and chicken carcasses (2). All isolates were cultured on tryptic soy broth or tryptic soy agar (Difco, USA) at 37°C for 24 h. Antimicrobial susceptibility test was determined by a broth micro-dilution method as described by Andrews JM (Andrews, 2001). Serial two-fold dilutions of β-lactam antibiotics, penicillin for *S. aureus*, ampicillin for *E. coli*, and amoxicillin for *S. typhimurium* were prepared within a dilution series of 0.0625–512 µg/ml in Mueller-Hinton II Broth (MHB) (Thermo, UK) on micro titer plates. The inoculum was prepared by suspension in MHB and adjusted the turbidity to 0.5 McFarland standard (approximately 6 log CFU/ml) and incubated at 37°C for 24 h. The interpretation of MIC was carried out in accordance with the CLSI guidelines (CLSI, 2010). Broth dilution acidimetric and chromogenic tests were performed by using a test kit and disk (Sigma, *Buchs, Switzerland*) according to the manufacturer's instructions as follows. Acidimetric method: Colonies of test organisms were suspended in penicillin-phenol red test reagent dispensed in 100 µl volume in microtitre wells. A change in color from purple pink to yellow within 15 minutes was regarded as positive production of *β* lactamase. Chromogenic method: Colonies of the test organisms were directly applied over a sterile moistened nitrocefin disc. A change in color from light yellow to deep red color within 15 seconds to 5 minutes was regarded as positive production of β lactamase. An iodometric test was performed as described by Samant and Pai (2012). *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *S.* Typhimurium ATCC 14028 were used as negative controls. Sensitivity and specificity were calculated by Shrestha and Rana (2014).

RESULTS AND DISCUSSION

Increasing antimicrobial resistance has become a major health problem both in veterinary and human medicine. β-lactams are the most widely used antibiotics for treatment of bacterial infections and β-lactamases are the important cause of resistance to these antibiotics (Samant and Pai, 2012). Table 1 shows the susceptibility and resistance of pencillin, ampicillin and amoxicillin against *E. coli*, *S. typhimurium* and *S. aureus* isolates. In this study, the resistance rate of penicillin in *S. aureus* was lower (75%) than that (95.91%) reported by Gajul *et al.* (2012) but higher than that (29.2 – 55.6%) reported by Samant and Pai (2012). Similarly, ampicillin resistance (44.44%) observed in *E. coli* isolates was much lower than that (100%) previously reported by Gajul *et al.* (2012).

In this study, we compared results of three β-lactamase detection methods (Table 2). Overall, the iodometric test yielded the highest positive results for β-lactamase detection with 44 positive (91.67%) results, followed by 32 (66.67%) positive results by chromogenic test and 23 (47.92%) positive results by acidimetric test. In a previous report by Gajul *et al.* (2012) acidimetric method yielded 208 (86.66%) positive results out of 240 total isolates for β-lactamase followed by 207 (86.25%) by iodometric method. Similarly, Samant and Pai (2012) reported β-lactamase activity in 42.8% of staphylococci by iodometric method and 42.5% by acidimetric method.

Table 1: Antimicrobial susceptibility of bacterial isolates against β-lactams by MIC test

Microorganism	β-lactam tested	Total isolates tested	Breakpoint (≥ µg/ml)	No. (%) of isolates	
				Susceptible	Resistant
S. aureus	Penicillin	16	0.25	4 (25.0)	12 (75.0)
E. coli	Ampicillin	18	32	10 (55.56)	8 (44.44)
S. typhimurium	Amoxicillin	14	32	9 (64.29)	5 (35.71)

Table 2: Comparison of β-lactamase detection by three different methods in veterinary isolates

Microorganism and susceptibility		No. (%) of isolates showing β-lactamase production by		
		Iodometric test	Acidimetric test	Chromogenic test
S. aureus (n=16)	Susceptible (n=4)	2	0	0
	Resistant (n=12)	12	12	12
Subtotal		14 (87.5)	12 (75.0)	12 (75.0)
E. coli (n=18)	Susceptible (n=10)	9	0	9
	Resistant (n=8)	8	6	8
Subtotal		17 (94.44)	6 (33.33)	17 (94.44)
S. typhimurium (n=14)	Susceptible (n=9)	8	0	0
	Resistant (n=5)	5	5	3
Subtotal		13 (92.86)	5 (35.71)	3 (21.43)
Total		44 (91.67)	23 (47.92%)	32 (66.67%)

Table 3: Results of three rapid β-lactamase test methods described in this study

Microorganism and test method	No. of isolates (*n*)				Sensitivity (%)	Specificity (%)
	True positive	False positive	True negative	False negative		
S. aureus (n = 16)						
Iodometric method	12	2	2	0	100	50
Acidimetric method	12	0	4	0	100	100
Chromogenic method	12	0	4	0	100	100
E. coli (n = 18)						
Iodometric method	8	9	1	0	100	10
Acidimetric method	6	0	10	2	75	100
Chromogenic method	8	9	1	0	100	10
S. typhimurium (n = 14)						
Iodometric method	5	8	1	0	100	11.11
Acidimetric method	5	0	9	0	100	100
Chromogenic method	3	0	9	2	60	100

It has been reported that rapid β-lactamase tests can give clinically relevant information about β-lactam resistance faster than disk diffusion or MIC tests (Shrestha and Rana, 2014). Table 3 shows the sensitivity and specificity of the three β-lactamase detection test methods and thereby the correlation between β-lactam susceptibility and β-lactamase production. Our results demonstrated that the sensitivities and specificities of the three different methods varied for *S. aureus*, *E. coli*, and *S. typhimurium* isolates. All three methods were found to be highly sensitive for *S. aureus* isolates. While the specificity of acidimetric and chromogenic methods was 100%, the specificity of iodometric method was only 50%. Thus, the results of acidimetric and chromogenic methods highly correlated with β-lactam resistance for *S. aureus* isolates tested. Among the *E. coli* isolates the sensitivity and specificity of iodometric and chromogenic were 100% and 10%, respectively, while those of acidimetric method were 75% and 100% respectively. Similarly, among the *S. typhimurium* isolates the acidimetric method gave most satisfactory results with the highest sensitivity (100%) and specificity (100%). Shrestha and Rana (2014) reported that chromogenic test was the most sensitive, acidimetric test was the least sensitive, and iodometric test was the most specific test during investigation of β-lactamase production in *S. aureus* isolated from two hospitals in Nepal and considered the chromogenic method to be the best method for the detection of β-lactamase.

Conclusions: These β-lactamase detection tests were quick, convenient, and can be valuable in rapid detection of β-lactamase production and prediction of β-lactam resistance in *E. coli*, *S. aureus*, and *S.* Typhimurium isolates. Our results showed that these test methods can be recommended for routine clinical use in veterinary laboratories for monitoring β-lactamase production among various microorganisms and to predict β-lactam antibiotic resistance.

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Authors' contributions: KJL, JWK, TWK conceived and designed the experiments; JWK, SHK, HCP, YJ performed the experiments; KJL, JYS, MG, JWK, TWK analyzed the data and MG, JWK, KJL wrote the manuscript. All authors critically revised the manuscript for important contents and approved the final version.

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