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Characterization for Staphylococcal enterotoxin B production and antibiotic susceptibility of *Staphylococcus aureus* isolated from Staphylococcul gastroenteritis (diarrhea)

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ABSTRACT

One hundred and seventy five diarrhoeal swab samples were collected twelve isolates (6.7%) were obtained and diagnosed as *Staphylococcus aureus*. Among 12 isolates, 8 (66.7%) were found to be positive for production of enterotoxin B. Antibiotic sensitivity of 12 *S. aureus* isolates were surveyed for susceptibilities to a panel of 20 antibacterial agents. *S. aureus* were 100% sensitive to Fusidic acid, while in the opposite direction, 100% resistancy was recorded for ampicillin and penicillin. Graded resistant was observed in the others, include: 66.7% for erythromycin, tetracycline, oxacillin and methicillin. In addition, the isolates showed resistance to trimetheprim (58.3%). The results of this study showed that a wide spread of enterotoxigenic and multidrug-resistant *S. aureus* isolates which isolated from diarrhea samples. The lowest pH value at which Staphylococcal enterotoxin B (SEB) production was observed 4 in the two isolates (SA4 and SA12). These isolates were able to produce SEB even at pH 10.5, which was the highest pH value among the isolates. The lowest temperature at which SEB production manifested was 20°C in four isolates (SA2, SA4, SA11 and SA12), while the highest temperature at which SEB was produced was 45°C, in three isolates (SA2, SA11 and SA12). SEB is produced within a wide pH range but the influence of temperature is an essential factor for the production of this toxin.

Keywords: Antibiotics, enterotoxin B*, Staphylococcus aureus.*

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INTRODUCTION

Representatives of the *Staphylococcus* genus are the most common pathogens found in hospital environments, and they are etiological agents for a large variety of infections (Cunha and Calsolari, 2008; Rong-hwa et al., 2010). *Staphylococcus aureus* (*S. aureus*) is an important major human pathogen capable of causing a wide variety of diseases, ranging from mild skin infections and food poisoning (FP) to life-threatening conditions, such as deep abscesses, osteomyelitis, pneumonia, necrotizing pneumonia, infective endocarditis, toxic shock syndrome (TSS), bacteraemia, septic arthritis, wound infections, pyogenic lesions, and sepsis. It can infect almost any organ, most notably bone tissue and cardiac valves (Arslan and Ozdemir, 2012; Kristlová et al., 2012; Argudin et al., 2013; Nada et al., 2012; Podkowike et al., 2013; Rahimi and Alian, 2013). *S. aureus* infections are difficult to control due to a combination of toxin mediated virulence, invasiveness and antibiotic resistance (Adwan et al., 2006; Qiu et al., 2010). Staphylococcal enterotoxins (SEs) are one cause of food poisoning in humans (Danielsen et al., 2013; Al-Jumaily et al., 2014). These enterotoxins were first characterized in 1959. SEs are thermostable and thus, may appear in food even if *S. aureus* is no longer present therein. To date, 23 SEs have been described (Chiao et al., 2013; Wua et al., 2013). SEs belong to a broad family of pyrogenic toxins

(Zhang et al., 2013). SEs are water-soluble, globular proteins consisting of a single chain Polypeptide, and structurally stable proteins with molecular weight ranging from 22 to 29 kDa. The common feature of SEs is high stability and resistance towards most proteolytic enzymes, such as pepsin or trypsin, allowing protection of their activity in gastrointestinal tract (Can and Celik, 2012; Clarisse et al., 2013). Gastrointestinal diseases are the most frequent causes of morbidity and mortality in developing countries (Vieira et al., 2001). *S. aureus* is considered one of the major borne pathogens throughout the world. *S. aureus* is considered the third most important cause of disease in the world among the reported foodborne illnesses (Souza et al., 2010). Since staphylococcal foodborne intoxication poses a threat for human health worldwide (Principato and Qian, 2014). Staphylococcal enterotoxin B (SEB) is a major virulence factor of staphylococcal diseases (Yarovinsky et al., 2005). As one of the major SEs causing food poisoning, SEB is extremely toxic with a half-lethal dose (LD50) of about 20 ng\kg and a half effective dose (ED 50) of about 0.4 ng\kg. SEA and SEB are two of the most important gastroenteritis causing agents. SEA and SEB are the most food poisoning (FP) agents (> 60%) in USA and England. Actually, SEB is the most important enterotoxin that causes gastroenteritis (Imani et al., 2010). According to previous studies, little as 100 ng of SEB may make a person ill with symptoms of classic food poisoning (Rong-Hwa et al., 2010). SEB is most likely to be associated with nosocomial infection (Nostro et al., 2002). Since SEB is an important agent of human disease, access to the methods of controlling diseases caused by SEB are important (Ataee et al., 2011; Yan et al., 2014). Antibiotic resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food. *S. aureus* has been reported to frequently show multiple antimicrobial resistance patterns (Alian et al., 2012). *S. aureus* is a versatile pathogen of humans that has evolved resistance to all classes of antimicrobials (Lozano et al., 2013). Multidrug-resistant *S. aureus* infections continue to increase and some strains respond to few, if any, conventional antibiotic therapies (Chen et al., 2012). Treatment of *S. aureus* infections can be challenging and expensive, especially with the high occurrence of antibiotic resistant infections (Spaulding et al., 2012). The threat of *S. aureus* is not only due to its distribution and pathogenicity, but also because of its ability to overcome antimicrobial agents (Sina et al., 2013).

Enterotoxin production by strains of *S. aureus* is affected by substrate quality, pH, temperature, atmosphere, sodium chloride, chemicals and other competing micro-organisms (Clarisse et al., 2013; Rahimi and Alian, 2013). The staphylococci grow in the temperature range between 7 and 48°C and produce SEs between 10 and 48°C, with optimum SEs producing temperature of 40 to 45°C. The optimum pH for toxin production is between 6.5 and 7.3 and the minimum pH that staphylococcal strains produce detectable SEs is reported to be 5.1. However once SEs are produced, they are resistant to low pH condition that easily destroys the bacteria that produced them, and retain the activity in the digestive tract after ingestion (Loir et al., 2003; Valero et al., 2009; Makita et al., 2012). Currently, there is limited information regarding the prevalence and antimicrobial susceptibility patterns of *S. aureus* isolation from clinical samples in Iraq. The aim of this study is to determine the prevalence rate, antimicrobial resistance and enterotoxigenecity of *S. aureus* isolates recovered from human clinical samples (diarrhea) in Babylon, Iraq; in addition, to influence environmental factors such as temperature and pH on the production of enterotoxin B from these bacteria.

MATERIALS AND METHODS

Isolation and identification of *S. aureus*

Diarrhoeal swab samples were collected from patients suffering from acute diarrhoea and gastroenteritis infection. The study was carried out for four months between December 2013 and March 2014 from the hospitals in Babylon governorate, 12 isolates of *S. aureus* were isolated from diarrhea samples out of 175 patients. Swabs were collected under aseptic conditions and inoculated into a tube containing 10 ml Tryptic soy broth. The broth was incubated at 37°C for 24 h then streaked from the enriched broth onto Mannitol Salt Agar plates and incubated at 37°C for 36 to 48 h. The colonies are circular, smooth and glistening (Sherein et al., 2009; Argudín et al., 2013).

All isolates were identified as *S. aureus* based on Gram staining (Gram-positive, non-spore forming cocci, arranged in form of single, pairs, short chains or in irregular clusters), beta-hemolytic activity on sheep blood agar, colonies are colorless to yellow. Biochemically, they are coagulase positive and are maltose fermenter to differentiate *S. aureus* from other Staphylococci. Confirmation of the genus, *Staphylococcus* was done by various biochemical tests including Catalase test, Oxidase test, Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, Motility Test, Citiate utilization, Urease, acid from different sugars and thermonuclease production (Viçosa et al., 2013). Single, well-isolated colonies with the typical appearance of *S. aureus* were subcultured, and identification was confirmed biochemically by API Staph. After the screening, the presumptive *S. aureus* isolates were stored at -80°C in Tryptic soy broth (TSB) plus 20% v/v glycerol (Zhang et al., 2013).

Antimicrobial susceptibility testing

All isolates were tested for susceptibility to a panel of twenty antimicrobials using disc agar diffusion method on Mueller Hinton agar .The antibiotic discs (antibiotic concentration in µg) from Oxoid are as follows: teicoplanin (Tei) (30), tetracycline (Te) (30), gentamicin (Gm) (10), oxacillin (Ox) (1), methicillin (Met) (5), vancomycin (Van) (30), ampicillin (AmP) (10), clindamycin (C) (2), erythromycin (E) (15), penicillin (P) (10 IU), cephalotin (Cep) (30), kanamycin (K) (30), fosfomycin (Fos) (30), chloramphenicol (Chl)

(30), trimethoprim (Tri) (2.5), fusidic acid (FA) (10), rifampicin (Rif) (5), Linezolid (Lzd) (30), ciprofloxacin (Cip) (5) and imipenem (I) (10) (Udo et al., 2006).

Two or three identical colonies were picked from the plate and transferred to the Mueller Hinton broth. The inoculum density was adjusted according to a 0.5 McFarland standard turbidity. Then a cotton swab dipped in the inoculum suspension and swabbed over the entire surface of agar. After incubation at 37°C for 24 h, zone diameter around the disk was measured and isolates were classified as susceptible, intermediately resistant and resistant as defined in Clinical and Laboratory Standards Institute (CLSI) (Arslan and Ozdemir, 2012).

Preparation of crude SEB toxin

The isolates were cultured in brain heart-infusion (BHI) broth supplemented with 1% yeast extract and incubated at 37°C for 18 h with rotating at 200 rpm. The culture supernatant was obtained by centrifugation at 3500 rpm for 20 min, and was passed through a membrane filter with a pore size of 0.45 µm (Millipore, Billerica, MA, USA). This solution was used as a source of SEB (Can and Celik, 2012).

Detection of SEB

Production of SEB was determined by reverse passive latex agglutination by using the commercial SET-RPLA kit (Oxoid) according to the manufacturer's recommendations. Briefly, microplate wells with a V-shaped bottom were inoculated with 25 µl of the supernatant and 25 µl latex sensitised with anti-enterotoxins. Standard toxins (provided by manufacturer, Oxoid Diagnostic Reagents) were used as positive controls and the occurrence of nonspecific reactions was tested by addition of 25 µl of the supernatant to 25 µl of control latex. The plates were covered with cellophane and homogenised in a micromixer for 3 min. After incubation for 20 to 24 h at environmental temperature, the results were recorded according to the agglutination pattern described by the manufacturer. Positive reactions (appear agglutination) were classified as (+), (++), and (+++), while formation of a pink bud was interpreted as a negative result (Argudin et al., 2012).

Effect of temperature and pH on production of SEB

A 24 h culture *S. aureus* (incubation at 37°C, blood agar) was inoculated into BHI broth (108 CFU/ml) (incubation 24 h at 37°C). The *S. aureus* suspension thus, prepared in the appropriate media (BHI was cultivated for 24h at various temperatures (5, 10, 15,20, 25, 30, 35, 40, 45 and 50°C) at a constant pH of 7.4 ± 0.2, or at differing pH (3 to 11) and a constant temperature of 37°C. The required pH values were achieved by modifying the pH of the BHI broth (pH 7.4) using (1 M of HCl and 0.2 M of NaOH). Subsequently, the samples were centrifuged at 3500 rpm for 20 min, the supernatant was pipetted off and centrifuging was repeated to remove the remaining cells (Kristlová et al., 2012). This supernatant was used as a source of SEB.

RESULTS

Isolation and identification of bacterial isolates

Twelve cultures of *S. aureus* recovered from 175 diarrhea samples (6.7 %). The identification of all *S. aureus* isolates was confirmed by conventional biochemical tests (Table 1).

Table 1. Biochemical characterization of *S. aureus* isolates isolated from diarrhea samples.

Biochemical test	Reaction
Catalase	+
Oxidase	-
Indole production	-
Nitrate reduction	+
Methyl red	+
Voges- Proskauer	+
Acid from sugar	
(a) Glucose	+
(b) Mannitol	+
(c) Maltose	+
(d) Lactose	+
(e) Raffinose	-
(f) Sucrose	+
Haemolysis	+
Coagulase	+
Thermonuclease	+
Motility test	-
Citiate utilization	-
Urease	+

SEB assay

All *S. aureus* isolates obtained in this study were investigated for their ability of SEB production by using the reverse passive latex agglutination (RPLA) method (Table 2). Among 12 isolates of *S. aureus*, 8 (66.7%) isolates had the ability to produce SEB.

Antimicrobial susceptibility

The resistance pattern of *S. aureus* isolates isolated from diarrhea samples to 20 antimicrobial agents tested in this study is shown in Table 3. The antimicrobial susceptibility profile revealed a high resistance of *S. aureus* to penicillin and ampicillin (100%). While the resistance to tetracycline, oxacillin, methicillin and erythromycin was 66.7 %. In addition, the isolates showed resistance to trimetheprim (58.3%). A low prevalence of resistance was detected for clindamycin (41.7 %), while kanamycin and ciproflaxacin (33.3%), the resistance to teicoplanin, chloramphenicol and imipenem was 25%. Few isolates

Table 2. SEB production from *S. aureus* isolates isolated from diarrhea samples by RPLA method.

Isolates	Degree of agglutination
SA1	+
SA2	+++
SA3	-
SA4	+++
SA5	++
SA6	++
SA7	-
SA8	+
SA9	-
SA10	-
SA11	+++
SA12	+++

Table 3. Antimicrobial susceptibility of *S. aureus* isolates.

Antimicrobial agent	Susceptible (%)	Intermediate (%)	Resistance (%)
Teicoplanin	9 (57)	0	3 (25)
Tetracycline	4 (33.3)	0	8 (66.7)
Gentamicin	9 (75)	1 (8.3)	2 (16.7)
Oxacillin	2 (16.7)	2 (16.7)	8 (66.7)
Methicillin	4 (33.3)	0	8 (66.7)
Vancomycin	9 (75)	1 (8.3)	2 (16.7)
Ampicillin	0	0	12 (100)
Clindamycin	7 (58.3)	0	5 (41.7)
Erythromycin	2 (16.7)	2 (16.7)	8 (66.7)
Penicillin	0	0	12 (100)
Cephalotin	11 (91.7)	0	1 (8.3)
Linezolid	11 (91.7)	0	1 (8.3)
Kanamycin	8 (66.7)	0	4 (33.3)
Fosfomycin	11 (91.7)	1 (8.3)	0
Chloramphenicol	8 (66.7)	1 (8.3)	3 (25)
Trimetheprim	5 (41.7)	0	7 (58.3)
Fusidic acid	12(100)	0	0
Rifampicin	10(83.3)	0	2 (16.7)
Ciproflaxacin	8(66.7)	0	4 (33.3)
Imipenem	9(75)	0	3 (25)

(16.7%) were resistant to gentamicin, vancomycin and rifampicin. A very low prevalence of resistance was detected for cephalotin and linezolid (8.3%); while susceptible ratio was high with fosfomycin (91.7%) and fusidic acid (100%).

Effect of temperature and pH on production of SEB

The lowest temperature at which SEB production manifested was 20°C in four isolates. The highest temperature at which SEB was produced was 40°C, in all studied isolates. Only three isolates (AS2, AS11 and AS12) can produce SEB in at 45°C (Table 4) and there was no any production of SEB at 5, 10, 15 and 50°C. The lowest pH value at which SEB production was 4 in isolates SA4 and SA12. These isolates were able to produce SEB even at pH 10.5, which was the highest pH value among the isolates studied (Table 5). SEB is produced within a wide pH range but the influence of

Table 4. SEB production by *S. aureus* isolates after 24h incubation in brain heart infusion (BHI) broth in dependence on temperature.

Temperature (°C)	Isolates							
	SA1	SA2	SA4	SA5	SA6	SA8	SA11	SA12
5	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
20	-	+	+	-	-	-	+	+
25	-	+	+	+	+	-	+	+
30	+	++	++	+	+	+	++	++
35	+	+++	+++	++	++	+	+++	+++
40	+	+++	++	++	+	+	++	++
45	-	+	-	-	-	-	+	+
50	-	-	-	-	-	-	-	-

*SEB was determined by RPLA method. Samples were analysed in duplicate.

Table 5. SEB production by *S. aureus* isolates after 24 h incubation in brain heart infusion (BHI) broth in dependence on pH value.

pH value	Isolates							
	SA1	SA2	SA4	SA5	SA6	SA8	SA11	SA12
3	-	-	-	-	-	-	-	-
3.5	-	-	-	-	-	-	-	-
4	-	-	+	-	-	-	-	+
4.5	-	+	+	-	-	-	+	+
5	-	+	++	+	-	-	++	+
5.5	-	+	++	+	+	-	++	+
6	+	+	++	+	+	+	++	++
6.5	+	+++	+++	++	++	+	+++	+++
7	+	+++	+++	++	++	+	+++	+++
7.5	+	+++	+++	++	++	+	+++	+++
8	+	+++	+++	++	++	+	+++	+++
8.5	+	++	+++	++	+	+	++	+++
9	-	+	++	+	+	-	+	++
9.5	-	-	++	-	-	-	+	++
10	-	-	++	-	-	-	+	++
10.5	-	-	+	-	-	-	-	+
11	-	-	-	-	-	-	-	-

*SEB was determined by RPLA method. Samples were analysed in duplicate.

temperature is an essential one for the production of this toxin.

DISCUSSION

Intestinal diseases of microbial origin are marked principally by diarrhea and sometimes by ulcero-inflammatory changes in the small or large intestine. Diarrhea posses a very serious problem where it is the leading cause of morbidity and mortality among children and adult. It ranks second only to respiratory diseases and is a major cause of morbidity among notifiable diseases in some part of the world (Yah et al., 2007). *S. aureus* represents the second cause of foodborne diseases after *Salmonella* spp. (Medveďová et al., 2009). *S. aureus* was isolated from diarrheic stool samples with a percentage of 6.7% (12 out of 175). Nearly similar results were previously reported by Flemming and Ackermann (2007) who isolated *S. aureus* from patients

with nosocomial diarrhea with a percentage of 7.3. Higher and lower results were previously reported by Gebreselassie (2002) whose result was 40.5%. However, lower percentage of 4.2% was reported by Bhalla et al. (2007). In addition, 3.1% was reported by Okolo et al. (2013), and 3.2% was reported by Yah et al. (2007).

Heat-stable enterotoxins, are a main cause of gastroenteritis. In this study, we determine the extent of enterotoxin-producing *S. aureus* in gastroenteritis infections of hospitalized patients. The results showed that 66.7% of these *S. aureus* produced SEB. Previous studies have reported variation in the prevalence of *S. aureus* and production of SEB. For example, the SEB producer among *S. aureus* isolated from clinical specimens 5% (Ataee et al., 2011). While isolates from bovine mastitis 21.6% (Al-Jumaily et al., 2014), in addition 25% produced SEB from skin infections (Imani et al., 2007). The prevalence of staphylococcal enterotoxin producing strains from human clinical samples differs among studies in different countries or in different areas of the same country. This might be due to differences in ecological origin of strains, the sensitivity of detection methods, number of samples and the type of clinical samples included in these studies.

Among the Gram-positive microorganisms, staphylococci are the most frequently resistant ones to antibiotics. *S. aureus* may develop resistance to various antimicrobial agents through different ways (Arslan and Ozdemir, 2012). The antimicrobial resistance profile of the tested *S. aureus* isolates to different antibiotics was analysed. The results in this study disagreement with other studies appeared no methicillin-resistant *S. aureus* strains were detected (Shareef et al., 2009; Alian et al., 2012; Yan et al., 2012). In this study, 75% of *S. aureus* isolates are vancomycin - susceptible. Whereas other study showed no resistance to vancomycin was found (Argudin et al., 2012; Can and Celik, 2012; Sina et al., 2013). In this study, 58.3% of *S. aureus* isolates are trimetheprim-resistant. Other studies showed none were resistant to trimetheprim (Shareef et al., 2009; Argudin et al., 2012). In this study, 66.7% of *S. aureus* isolates are ciproflaxacin- susceptible. Whereas other studies showed no resistance to ciprofloxacin was found (Alian et al., 2012; Argudin et al., 2012). In this study, 25% of *S. aureus* isolates are imipenem–resistant. The results in this study disagreement with other studies onted all strains were susceptible to imipenem (Arslan and Ozdemir, 2012). These results are similar to the results of other study by Can and Celik (2012) which showed that 25% of *S. aureus* isolates isolated from turkish cheeses are teicoplanin–resistant. While dissimilar with Can and Celik (2012) reported that 25 and 33.3% of isolates were resistant to tetracycline and clindamycin, respectively. Whereas all isolates were susceptible to gentamicin. On the other hand, 16.6% of isolates were more resistant to oxacillin and methicillin. Also, 50% of isolates were resistant to ampicillin and erythromycin.

The results in this study are dissimilar to the results by Jaber (2011) which found that 75% of isolates were resistant to gentamicin. Yan et al. (2012) found that 28.8, 96.2 and 3.8% of *S. aureus* strains isolated from clinical specimens were resistant to tetracycline, penicillin and rifampicin respectively. The results in this study are in disagreement with Yan et al. (2012) which noted that the clindamycin and erythromycin resistance were observed less frequently 7.7%. The results in this study are similar to other study by Pereira et al. (2009) which found a small percentage of the strains demonstrated resistance to gentamicin. On the opposite site, the results in this study are dissimilar to another study by Shareef et al. (2009) which found that 100% of *S. aureus* strains isolated from chicken were susceptible to tetracycline and 80% of strains were resistant to chloramphenicol. Sina et al. (2013) showed that 25% were resistant to oxacillin. These results in this study are similar to the results by Sina et al. (2013) which showed that all of the strains isolated from skin infections were resistant to benzyl penicillin also fosfomycin and fusidic acid was active against some of the strains.

These results in this study are dissimilar to the results by Ackermann et al. (2005) found all *S. aureus* strains isolated from diarrhoea were susceptible to oxacillin. The results presented here are similar to a previous study by Schlievert et al. (2010), which showed that more than 50% of hospital-associated *S aureus* strains are methicillin-resistant. Methicillin resistance is not a direct factor that increases virulence of *S aureus*, but rather functions indirectly to influence production of secreted virulence factors, and clearly makes clinical management of staphylococcal infections more difficult. Recently, even a small number of vancomycin-resistant strains have been isolated from patients. Arslan and Ozdemir (2012) noted that 93% of *S. aureus* strains isolated from clinical specimens were susceptible to linezolid. To prevent the unnecessary use of β-lactams and to achieve effective therapy, isolation of the microorganisms and determination of antimicrobial susceptibility is essential before the start of any treatment. The use of antimicrobial agents in the treatment of diarrhea diseases has greatly improved the quality of life among residents. However, the problems associated with microbial resistance in diarrhea patients still pose a challenge to public healthworks. Also, a call to regulate the use of antimicrobial may be necessary to reduce the resistance to drugs. In this research, there was a high level of antimicrobial resistance or multidrug resistant isolates, a possible monitoring of antimicrobial susceptibilities of important pathogen *S. aureus* may be important in helping the choice of antibiotic to control infections in human medicine and veterinary practice.

The production of SEs is dependent upon many external factors. The most important factors include pH

and temperature (Kristlova et al., 2012). Rajkovic (2012) showed that both growth of *S. aureus* and SEs production can occur even in the final product at 22 ºC and 37 ºC, but not at 12°C. Valero et al. (2009) reported that the growth of *S. aureus* was observed at pH = 4.5, demonstrating the ability of *S. aureus* to grow under acidic pHs. Observation obtained from the replicates tested showed that growth was higher at pH 7.0 than at pH 7.5. This fact is especially important when predicting the SEs production, because the optimum pH and temperature for SEs production are generally slightly higher than that for growth; 7.0 to 8.0 pH values and above 10°C. Cunha and Calsolari (2008) analyzed the production of SEB by coagulase-negative staphylococci (CNS) at different temperatures, pH values and most production occurred at a temperature of 39.4°C with pH 7.0. When the temperature was increased to 41°C at pH 4, inhibition of enterotoxin production was observed. With regard to pH, it observed that the production of enterotoxins did not occur in pHs higher than 9.0 or lower than 5.0, and at pH 8.0, a 50% decrease in their production takes place. Optimal pH for SEB production is 6.8.

Other study had investigated that the highest temperatures range for SEB production were 35 to 45.5°C (Schmitt et al., 1990). In this study, the production of SEB toxin was observed at 45°C, but at temperature 50°C production of the toxin was no longer manifested. If the growth of *S. aureus* is inhibited by low pH, we may presume that also SEs production will be limited. Production of SEB still occurred at pH 4 to 10.5. Moreover, Kristlová et al. (2012) found that strains of *S. aureus* may grow and produce SEH in media with pH 4.3 to 10.6. In this study, it was determined that temperature has the greatest influence on the production of SEB, since at temperatures lower than 20°C its production was not manifested. *S. aureus* is, however, capable of producing SEB within a wide pH range, which was confirmed for other SEs as well.

Conclusion

The results of this study showed the wide spread of enterotoxigenic and multidrug-resistant *S. aureus* isolates isolated from diarrhea samples. Hence, a diagnostic microbiology laboratory should be equipped to detect enterotoxigenicity in addition to bacterial identification. In this way, new methods can be designed to treat infections better, prevent undue use of antibiotics, and prevent the emergence of antibiotic resistance. This research has the significance to learn more about enterotoxins and which factors contribute to their production as well as to further understanding of their toxicological properties.

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