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Prevalence of Various Enterotoxins among Clinical *Staphylococcus aureus* Strains Isolated from Food Borne Poisoning Patients

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The main objective of this study was investigation of the prevalence of various enterotoxins among clinical *Staphylococcus aureus* strains isolated from food borne poisoning patients. A total of forty two clinical *S. aureus* strains were isolated from patients with food poisoning symptoms in the King Saud Medical Complex (Riyadh, Saudi Arabia). Detection of *S. aureus* enterotoxins were performed using two methods; including commercially available SET-RPLA serological kits; and PCR amplification of various enterotoxins genes. Comparison between detection of enterotoxins production by SET-RPLA and PCR amplification of enterotoxins genes approaches indicated that *sea* gene was detected in 64% (27/42) of the tested *S. aureus* strains, while SEA was detected in 45% (19/42) using SET-RPLA. For *seb* gene and SEB production, it was detected in 85% (41/42) and 38% (16/42) using PCR and SET-RPLA techniques, respectively. In addition, 98% (41/42) of the strain showed *sec* gene using PCR, while only 31% (13/42) were positive for SEC. Interestingly, while SED was detected in only 16% (7/42) using SET-RPLA, it was detected in 88% (37/42) of the tested *S. aureus* strains using PCR. These results clearly demonstrated that detection of staphylococcal enterotoxins by PCR was much more efficient than SET-RPLA in *S. aureus* strains. Finally, the antibiotics susceptibility of the isolated clinical *S. aureus* strains (n=42) were determined. The results indicated that some strains were resistant to some antibiotics including ciprofloxacin (31%, n=13), moxifloxacin (29%, n=12), azithromycin (22%, n=9) and tetracycline (5%, n=2). In addition, three strains (7%) were resistant to methicillin.

Keywords: *Staphylococcus aureus*, Enterotoxins, SET-RPLA, Food poisoning, Antibiotics susceptibility.

Staphylococcus aureus is one of the major bacterial pathogens, which cause various clinical infections and food-poisoning cases, and is considered as the world's third most important cause of food-borne illnesses (Pereira *et al*., 2009). It is a common pathogen associated with serious community and hospital acquired diseases and has for long time been considered as a major problem of Public Health(Chiang *et al* 2008). Such infections are often acute and pyrogenic and, if untreated, may spread to the surrounding tissues and metastatic sites (El-Huneidi *et al*., 2006). *S.aureus* is an important food-borne pathogen dueto its ability to produce a wide range of extracellular protein toxins and other virulence factors that contribute to the pathogenicity of the organism. Of particular relevance to the food processing

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industry is the ability of some *S. aureus* strains to produce heat stable enterotoxins that cause staphylococcal food poisoning (SFP), which considered to be one of the leading causes of all food-borne diseases(Boerema *et al*., 2006; Shimamura *et al*., 2009). In the last few decades SFP has been reported as third cause of food-borne illnesses in the world. In U.S. the annual number of SFP is 185,000 cases, and in Europe *S. aureus* caused 5.1% of the food-borne outbreaks between 1993 and 1998, however, the real incidence of SFP is underestimated (Shimizu *et al*., 2000; Anvari *et al*., 2008; Huong *et al*., 2010). SFP is a mild intoxication occurring after the ingestion of food containing from 20 ng to < 1 µg of staphylococcal enterotoxin (SE). SFP symptoms appear within a few hours after ingestion of contaminated food, the symptoms are depending on individual susceptibility and toxic dose ingested which include nausea, abdominal cramps, diarrhea and a characteristic projectile vomiting (Oranusi*et al*., 2006; Morandi*et al*., 2007; Huong *et al*., 2010).

Although this facultative anaerobic bacterium possesses a wide spectrum of virulence properties, including extracellular proteins like adhesins, invasins, and hemolysins, staphylococcal enterotoxins (SEs) are recognized as the most important factors for its pathogenicity (Chiang *et al* 2008; Pereira *et al*., 2009; Rahimi *et al*., 2010). Staphylococcal enterotoxins (SE) are a group of single chain low-molecular weight proteins (26900–29600 Da) that are usually divided into the classic SEA to SEE and newly described (SEG to SER and SEU) enterotoxins (Letertre *et al*., 2003; Fueyo *et al*., 2005; El-Huneidi *et al* 2006; Morandi *et al*., 2007, Rahimi *et al*., 2010). Various methods have been developed for the detection of the enterotoxins and related genes. Reverse phase latex agglutination (SET-RPLA), and Staphylococcal enterotoxin ELISA (SET-EIA); and TECRA kits, were usually used. However, recently, polymerase chain reaction (PCR) proved to be a useful tool for the rapid and reliable detection of the enterotoxins genes (Chapaval*et al*., 2006; Chiang *et al* 2008; Rahimi *et al*., 2010).

There is no enough research on the staphylococcal enterotoxins of strains isolated in Saudi Arabia particularly at the molecular level, hence, this study aimed to identify and characterize the types of staphylococcalenterotoxins produced by clinical *Staphylococcus aureus*strains isolated from patients with food borne poisoning symptoms, in addition to investigation of antibiotics resistance of such important pathogens.

MATERIALS AND METHODS

Clinical samples collection and bacterial strains identification

A total of forty two clinical *Staphylococcusaureus* isolates included in this study were isolated from patients with food poisoning symptoms in the King Saud Medical Complex (Riyadh, Saudi Arabia), during the period between October 2009 and April 2010. The specimens were collected under sterile conditions using sterile cotton swabs, syringes and transport media, and were transferred to the laboratory in cold box within 1 to 2 h. The isolated clinical bacterial strains were identified using conventional biochemical tests, API 20NE (Biomérieux) and VITEK®-2or MicroScan Walk-Away® microbial identification systems(Dade Behring, CA), as previously reported (Dalla-Costa *et al*., 2003; Jeon *et al*., 2005; Villalon *et al*., 2011).

Haemolysin production

Haemolytic activity of the isolated clinical *S. aureus*strains was determined on Columbia Blood Agar Base (Oxoid, UK) containing 5% defribrinated horse blood. The inoculated plates were incubated aerobically at 37°C for 24-48 h. Clear zonesaround the colonies indicated haemolysin production.

Screening for the production of different enterotoxins

Detection of *S.aureus* enterotoxins were performedusing two methods; (i) Serological method, using commercially available SET-RPLA; and (ii) Molecular method, using PCR amplification of the enterotoxins genes.

Detection of enterotoxins production Reverse Passive Latex Agglutination

Production of various staphylococcal enterotoxins including SEA, SEB, SEC and SED were carried out using Reverse Passive Latex Agglutination Assay (SET-RPLA), (Huong *et al*., 2010). First, the isolated clinical *S.aureus*(n=42) were grown in Brain Heart Infusion broth (Difco) for 18 h at 37 °C. The broth culture was then centrifuged for 20 min at 10 000 rpm and the

supernatantswerecollected and filtered through 0.45-µm filters. Then, the filtrateswere tested for the presence of various staphylococcal enterotoxins by reversed passive latex agglutination assay (RPLA) using commercial SET-RPLA kits (Denka Seiken, Japan) following the manufacturer's instructions.

PCR detectionofenterotoxins genes of *Staphylococcusaureus*

Polymerase chain reaction (PCR) was used for detection of the staphylococcal enterotoxins in the isolated clinical *S. aureus*strains (n=42) strains).The primers specific for different genes used in this study are presented in Table 1. *S. aureus*strains (n = 42) were grown in 5 ml broth medium at 37ºC for overnight. Then,the cells biomass were collected by centrifugation at 7000 × *g* for 10 min, and washed twice using sterile distilled water. Total bacterial DNA was extracted from the cells using DNeasy blood and tissue kits (Qiagen) following the manufacturer's instructions. Conventional PCR was performed for detection of different SEs genes in the isolated clinical *S. aureus*including *sea, seb, sec* and*sed*genes.PCR amplification was performed in a final reaction volume of 50 µl and the reaction mixtures contained 25 µl of GoTaq® Green Master Mix (2X), (Promega, cat no. 7122), 1 µl of upstream primer (10 µM), 1 µl of downstream primer (10 µM), 5 µl deoxyribonucleic acid (DNA) template (200 ng) and 18 µl of nuclease-free water. The PCR reaction was run for 35 cycles in a DNA thermal cycler under the following thermal profile: Initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, primers annealing at 52°C for 1 min and extension at 72°C for 1.5 min. The final cycle included extension for 10 min at 72°C to ensure full extension of the products. Then, the PCR products were analyzed using agarose gel electrophoresis.

Antimicrobial agent and determination of MICs

Susceptibility of the isolated non-repetitive clinical *S. aureus*strains toward various antibiotics (n = 17) was investigated using agar disc diffusion method (Poirel *et al*., 2007; Bonnin *et al*., 2011). All bacterial strains were sub-cultured in fresh Mueller-Hinton agar plates for 18 h at 37°C. After the incubation period, the cells were collected using sterile loop and suspended in sterile saline solution (0.9% NaCl) to be equivalent to 0.5 McFarland standards. The cells suspensions were inoculated into Mueller-Hinton agar plates (Difco), using sterile cotton swabs, and various antibiotic discs were placed (in duplicate) carefully on the agar plates surfaces and incubated for 24 to 48 h at 37°C. Inhibition zone diameter were evaluated according to clinical and laboratory standard institute (CLSI) guideline (Peleg *et al*., 2008, Poirel *et al*., 2010). The MICs of various antibiotic (n = 17) against the isolated *S. aureus*strains (n = 42) were determined using MicroScan Walk-Away® automated system according to the manufacturer's instructions.MIC values were determined as the lowest concentration of antibiotic able to inhibit the bacterial growth and the results wereinterpreted as recommended by CLSI (Bertini *et al*., 2007; Peleg *et al*., 2008).

RESULTS AND DISCUSSION

Isolation and identification of clinical *S. aureus*strains

Enrichment and isolation of *S. aureus*from the collected clinical specimens,from patients with food poisoning symptoms in the King Saud Medical Complex (Riyadh, King Saudi Arabia), resulted in isolation of 42 non-repetitive clinical

Table 1. List of primers used in this study

Gene	Primers	Sequence	Reference
Sea	*Sea*-F	GCA GGG AAC AGC TTT AGG C	Pereira *et al*., 2009
	Sea-R	GTT CTG TAG AAG TAT GAA ACA CG	Pereira *et al*., 2009
Seb	*Seb*-F	GTA TGG TGG TGT AAC TGA GC	Silva *et al*., 2005
	Seb-R	CCA AAT AGT GAC GAG TTA GG	Silva *et al*., 2005
Sec	*Sec*-F	CTT GTA TGT ATG GAG GAA TAA CAA	Pereira *et al*., 2009
	Sec-R	TGC AGG CAT CAT ATC ATA CCA A	Pereira *et al*., 2009
Sed	*Sed*-F	GTG GTG AAA TAG ATA GGA CTG C	Pereira *et al*., 2009
	Sed-R	ATA TGA AGG TGC TCT GTG G	Pereira *et al*., 2009

bacterial strains. All isolates were non-motile gram positive cocci, non-spore forming bacteria, with golden yellow colonies. The results of the biochemical tests indicated that all strains were positive for catalase, DNase and coagulase, in addition to fermentation of mannitol salt. The API kit identified the isolated bacterial strains as *S. aureus*. All of the biochemical properties were consistent with those reported for typical strain of *S. aureus*(Chapaval*etal*., 2006; Oranusi*etal*., 2006; Randa*etal*., 2006; Rahimi *et al*., 2009; Rahimi and Safai, 2010; El-Jakee*etal*., 2010). Moreover, the bacterial identity was confirmed using automated MicroScan Walk-Away®.Analysis of the haemolytic activity of the isolated *S. aureus* strains (n=42), indicated that 93% of the strains had hemolytic activity. 54% of the strains (n=21) showed â-hemolysis, whereas 46% (n=18) showed β-hemolysis, and 3 isolates (7%) were non haemolytic.α -haemolytic strains of *S. aureus*are known to be more of human biotype and more toxigenic than α-haemolytic strains which are more abundant in animal and less toxigenic to human (Oranusi *et al*., 2010).

Screening for the production of different enterotoxins

Detection of *S.aureus* enterotoxins were performedusing two methods; (i) Serological method, using commercially available SET-RPLA; and (ii) Molecular method, using PCR amplification of the enterotoxins genes.

Detection of staphylococcal enterotoxins production by SET-RPLA

The production of the major classical staphylococcal enterotoxins (SE's), including SEA, SEB, SEC, and SED by the isolated clinical *S.aureus* strains (n=42) were detected serologically using SET-RPLA assay. The results indicated that all *S. aureus* strains (n=42) showed SE's production: 69% (n=29) of the tested strains showed production of at least one SE, whereas two different SE's were detected in 31% (n=13) of the tested strains. Out of the 42 isolated *S. aureus* strains, 19 strains (45%) produced SEA, 16 strains (38%) produced SEB, 13 strains (31%) produced SEC, and 7 strains (16%) produced SED (Fig. 1). In addition, some strains produced only one enterotoxin, that 7 strains produce only enterotoxin A "SEA", 4 strains produce only enterotoxin B "SEB", 11 strains produce only enterotoxin C "SEC", and 7 strains produce only enterotoxin D "SED" (**Figure 2**). Moreover, it was found that some strains produce two different SE's, that 11 strains produced SEA

Table 2. Results of PCR detection of staphylococcal enterotoxins for each of clinical isolated of *S. aureus* (42 strains)

No. strains	Enterotoxins gene(s)			
	sea	*seb*	*sec*	*sed*
2491	-	+	+	+
2492	-	+	+	-
2339	-	+	+	+
2343	+	+	+	+
2344	+	+	+	+
2535	+	+	+	+
2536	+	+	+	+
2547	+	+	+	+
2548	+	+	+	+
2351	+	+	+	+
2411	+	+	+	+
2448	+	+	+	-
2428	+	-	+	+
2724	-	+	+	+
2664	+	+	+	+
2660	+	+	+	+
2579	+	+	+	+
2384	-	+	+	+
2340	-	+	+	+
2607	+	+	+	+
2668	+	+	+	+
2500	+	+	+	-
2519	+	+	+	+
2520	+	+	+	+
2475	+	-	+	+
2476	-	+	+	-
2412	-	+	-	+
1743	+	+	+	+
674	+	+	+	+
675	+	+	+	+
691	+	+	+	-
741	-	+	+	+
746	+	+	+	+
747	-	-	+	+
749	-	+	+	+
750	+	-	+	+
752	-	+	+	+
753	-	+	+	+
757	+	-	+	+
779	-	+	+	+
781	+	+	+	+
2305	-	-	+	+
Total	27 (64%)	36 (85%)	41 (98%)	37 (88%)

and SEB, one strain produced SEA and SEC, and one strain produce SEB and SEC (Fig. 3).Prevalence SE's production among the isolated *S. aureus* strains were relativelysimilar to that reported by Kérouanton *et al* (2002) for food poisoning outbreaks in France by *S. aureus*. However, it washigher than that reported by Asghar *et al* (2006) in *S. aureus*strains isolated inMakka city (Saudi Arabia), where SEA, SEB, and SEC production were detected in 27%, 36% and 36% of the tested strains (n=54), respectively, using SET-RPLA method. Prevalence of SEA was relatively similar to that reported in Italy by Morandi *et al* (2007), who reported high percentage of SEA (42%) production. However, higher distribution of SEA production (57.9% of the tested strains) was also reported in Japan (Omoe *et al*., 2005). SEA is the most common enterotoxin recovered from food-poisoning outbreaks in the US followed by SED and SEB (Kwon *et al*., 2004). Furthermore, In the present study only one strain produced SEA and SEC, which is very similar to that reported in Korea Moon *et al*., 2007). SED (16%) was the minor ones found in isolates from patients with food-poisoning cases which are also relatively similar to that reported in Egypt by El-Seedy *et al*.(2010). This percentage was lower than that reported in Italy by Morandi*et al*. (2007), who reported detection of SED in 42% of the test strains. However, in another study in Germany SED was much less (0.75%), (Klotz *et al*., 2003).

PCR detection of enterotoxins genes

PCR was used for detection of classical staphylococcal enterotoxins genes (*se*'s) in the isolated clinical *S.aureus* (n=42), including *sea*, *seb*, *sec* and *sed*. This was carried out by extraction of total bacterial DNA of the *S.aureus* strains (n=42) followed by PCR amplification of various *se*'sgenes using specific primers presented in Table 1. The results indicated that various *se*'sgenes were detected in all of the tested *S. aureus* strains (n=42) that 47.6% of the tested *S. aureus* strains (n=20)

Table 3. Summary of susceptibility of the isolated clinical *S. aureus* strains (n=42) toward various antibiotics (n=22). Susceptibility testing was carried out using automated MicroScan Walk-Away® system

Antibiotic	Susceptibility					
	Sensitive		Intermediate		Resistant	
	%	No.	%	No.	%	No.
Amox/K Clav	100	42	0	0	0	0
Ampicillin	100	42	0	0	0	0
Azithromycin	76	32	2	1	22	9
Cefazolin	100	42	0	0	0	0
Ceftriaxone	100	42	0	0	0	0
Cefuroxime	100	42	0	0	0	0
Ciprofloxacin	62	26	7	3	31	13
Clindamycin	86	36	14	6	0	0
Erythromycin	81	34	19	8	0	0
Fusidic acid	93	39	7	3	0	0
Gentamicin	100	42	0	0	0	0
Linezolid	100	42	0	0	0	0
Moxifloxacin	69	29	2	1	29	12
Mupirocin	100	42	0	0	0	0
Nitrofurantion	100	42	0	0	0	0
Oxacillin	93	39	0	0	7	3
Penicillin	100	42	0	0	0	0
Rifampin	100	42	0	0	0	0
Synercid	98	41	2	1	0	0
Teicoplanin	100	42	0	0	0	0
Tetracycline	95	40	0	0	5	2
Vancomycin	98	41	2	1	0	0

were positive for all enterotoxins genes, 40.5% of the strains (n=17) were positive for three enterotoxins, and 11.9% of *S. aureus* strains (n=5) were positive for two enterotoxins (Table 2). As shown in Fig. 4, *sec* gene was the most frequent gene (n=41; 98%), followed by *sed* (n=37; 88%), *seb*(n=36; 85%), and *sea* gene (27; 64%), respectively. In addition, there was significant variation among the strains regarding the number *se*'s genes detected in each strain. This results in agreement with that reported by Silva *et al* (2005), who reported that *sec* was the most frequently produced toxin; also, similar to that with that reported by El-Jakee *et al*., who reported highest detection of *sec* in the tested strains (El-Jakee*et al*., 2010). However, it was higher that reported by Anvari *et al*., that *sec* where reported in 46% of tested strains (Anvari *et al*., 2008). However, other investigators have reported that the *sec* gene is the most frequent among strainsin Europe and the

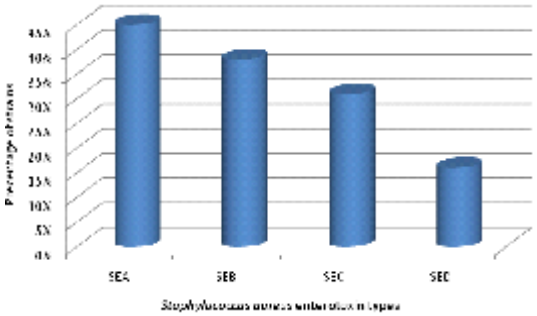

Fig. 1. Percentage of various staphylococcal enterotoxins (SE's) in the isolated clinical *S. aureus* (n=42), detected by SET-RPLA method

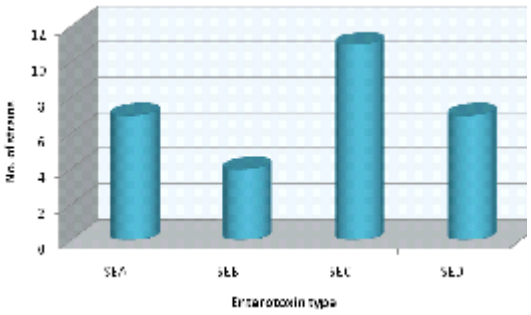

Fig. 2: *S. aureus* strains that produce only one enterotoxin among the isolated clinical *S. aureus* (n=42), detected by SET-RPLA method

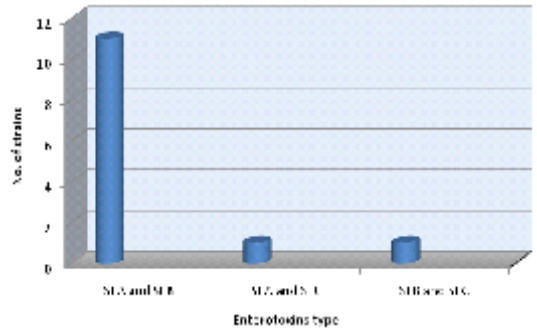

Fig. 3. Percentage of *S. aureus* strains that produce two different enterotoxins, detected by SET-RPLA method

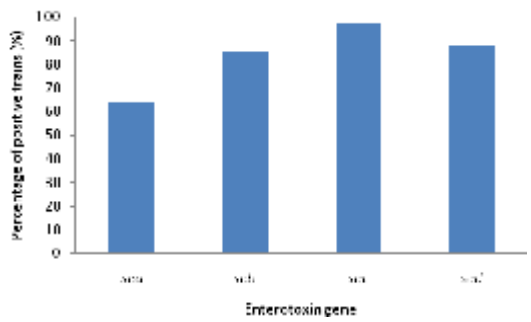

Fig. 4. PCR detection of SE'S gene in the isolated clinical *S. aureus* (n=42)

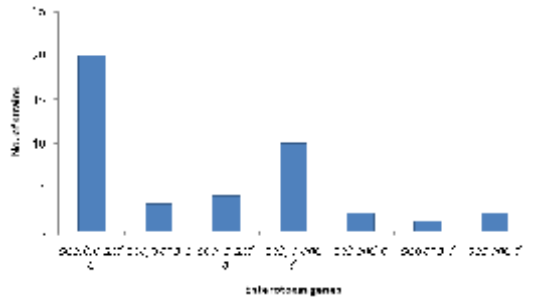

Fig. 5. *S. aureus* strains that contain more than one staphylococcal enterotoxins (SE's) gene

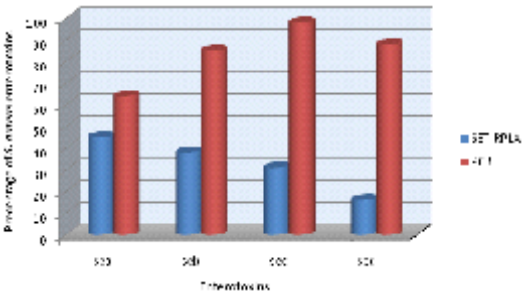

Fig. 6. Comparison between SET-RPLA and PCR method for detection of staphylococcal enterotoxins (SEs) in the isolated *S.aureus* (n=42)

USA (Sharma *et al*., 2000& Larsen *et al*., 2002).

The most frequent *se* gene profiles was *seb-sec-sed* (n=10), followed by *sea-sec-sed* (n=4), *sea-seb- sec* (n=3), *seb-sec* (n=2),*sec-sed* (n=2), and *seb-sed* (n=1), respectively (Figure5). Therefore, our results show that the *sec* gene is dominant in *S.aureus* strains linked to staphylococcal food poisoning (SFP) cases in Saudi Arabia. This was in contrast to the results reported about SFP food poisoning outbreaks in France, where *sea* gene was the dominant gen with *sea–sed* as most frequent *se* gene profile. In Korea, the *sea* gene was also dominant and frequently associated with *seh* or seg–sei in strains linked to 40 SFP outbreaks (Chapaval*et al*., 2006). In Japan, the predominant *se*'s produced by *S. aureus* strains from 129 SFP outbreaks are *sea*, *sea-seb* and *seb* (Shimizu *et al*., 2000). It was reported that most ovine biotype strains and isolates from caprine bulk milk being *sec* producers (Jorgensen *et al*., 2005), therefore, the staphylococcal food poisoning reported here could be due to consumption of meat and dairy products contaminated with *S. aureus* strains.

Comparison between PCR detection of enterotoxins genes and SET-RPLA techniques

Comparison between detection of enterotoxins production by SET-RPLA techniques and PCR amplification of enterotoxins genes approach indicated that *sea* gene was detected in 64% (27/42) of the tested *S. aureus* strains, while SEA was detected in 45% (19/42) using SET-RPLA (Figure 6). For *seb* gene and SEB production was detect in 85% (41/42) and 38% (16/42) using PCR and SET-RPLA techniques, respectively. In addition, 98% (41/42) were showed *sec* gene using PCR, while only 31% (13/42) were positive using SET-RPLA. Interestingly, while SED was detected in only 16% (7/42) using SET-RPLA, it was detected 88% (37/42) of the tested *S. aureus* strains using PCR, which indicated that detection of enterotoxins by PCR was much more than SET-RPLA in *S. aureus* strains (Figure 6). These results were similar with another report described by (Klotz *et al*., 2003), who reported that detection of enterotoxins by PCR was more efficient than SET-RPLA in *S. aureus* strains. Also, the results in this work were in agreement with another report, who reported PCR detection of enterotoxins was more effective than SET-RPLA (Atanassova *et al*., 2001 & Loncarevia *et al*., 2005). Other reports in Japan and Egypt, the SET-RPLA and PCR were very similar with results in this study (Shimamura and Murata 2009 & El-Seedy *et al*., 2010).

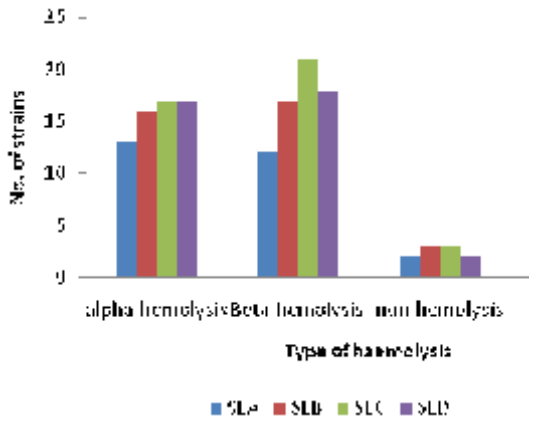

Fig. 7. Relationship between blood haemolytic activity and staphylococcal enterotoxins production in the isolated clinical *S. aureus* strains (n=42)

Relationship between staphylococcal enterotoxins genes and hemolysis

Analysis of the of blood haemolysis by the isolated *S. aureus* clinical strains indicated that 92.3% of the strains (n=39) were hemolytic, while only 3 strains (7.7%) were non-hemolytic *S. aureus* (Figure 7). These results are very similar with results reported by El-Jakee in Egypt (El-Jakee*et al*., 2010). It was found that 21 strains (50%) showed β-hemolysis, and 18 strains (43%) were α-hemolysis, in addition to 3 strains (7.7%) that were non-haemolytic. The result for β-hemolysis in this work was similar to that reported by Akineden *et al* (2001), who reported that 49% of the tested strains were β-hemolytic; however in his study; α-hemolysis was higher (24%). However, the percentage of β-hemolytic *S. aureus* in present study was lower than that reported by Islam *et al*. (2007), who recorded 89.3% of the tested strains were β-hemolytic. As shown in Figure 7, analysis of the relationship between enterotoxins prevalence and haemolysis properties indicated that 44.5 % of the strains showing *sea* were beta-hemolytic, 48.1 % were α-hemolytic, and 7.4 % were non-hemolytic. 47.2 % of the strains showing *seb* were β-hemolytic, 44.5% were β-hemolytic, and 8.3 % were non-hemolytic. 51.2 % of the strains showing *sec* were beta-hemolytic, 41.5% were contained 21 (51.2 %) strains were β-hemolysis; 46 % were β-hemolytic, and 5.4 % were non-hemolytic. In addition, 48.6 % of the strains showing *sed* were

β-hemolytic, 46 % were contained 21 (51.2 %) strains were α-hemolysis; 46 % were α-hemolytic, and 5.4 % were non-hemolytic.

Antibiotic susceptibility

Susceptibility of the isolated clinical *S.aureus* (42 strains) toward various antibiotics (n=22) were determined using disc diffusion assay and automated MicroScan Walk-Away® system. The results presented in Table 3 indicated that all strains were methicillin sensitive except 3 strains (7%) that were resistant to methicillin (MRSA). In addition, some strains were resistant to some antibiotics including ciprofloxacin (31%, n=13), moxifloxacin (29%, n=12), azithromycin(22%, n=9) and tetracycline(5%, n=2). However, some strains were intermediate for some antibiotics, including erythromycin(19%, n=8), clindamycin(14%, n=6), ciprofloxacin(7%, n=3), fusidicacid (7%3), synercid(2%, n=1) and vancomycin1 (2%, n=1). This result is relatively similar to that reported by El-Seedy *et al* (2007). However, the results of MRSA resistant strains was lower than reported by Zahan *et al*., in Bangladesh, who recorded that 17.5% of the tested strains were MRSA resistant (Zahan*et al*., 2008). However,the result MRSA resistance in present study is very similar with that reported by Islam *et al*. (2007), who recorded 7.1% of the tested strains were MRSA.

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