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Should we leave the paper currency? A microbiological examination

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ABSTRACT

Objetives. Pathogens can be transmitted to banknotes due to the personal unhygienic habits. The aim of study was to find the possible pathogens on the banknotes circulating in the market and also to present their antibacterial resistance and their various virulence factors using genotypic and phenotypic methods.

Material and methods. A total of 150 samples of banknotes were randomly collected between August 2017 and March 2018. VITEK systems were used for identification and antimicrobial susceptibility testing respectively. Antimicrobial resistance genes (*mecA*, *van*, extended-spectrum β-lactamase [ESBL] and carbapenemases) and staphyloccoccal virulence genes (staphyloccoccal enterotoxins [SEs], *pvl*, and *tsst-1*) were determined using with real-time PCR.

Results. *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Enterococcus* spp., Gram-negative enteric bacteria, non-fermentative Gram-negative bacteria and *Candida* spp. were detected 48%, 54.7%, 56%, 21.3%, 18.7%, and 4%, respectively. Methicillin-resistant *S. aureus*, vancomycin-resistant enterococci and ESBL producing Gram-negative were found 46.8%, 1.3%, and 28.7%, respectively. *Pvl*, *tsst-1*, and SEs genes were found in a 2.8/4.9%, 1.4/1.2%, and 100/87.8% of the *S. aureus*/CoNS strains, respectively. The *sea* gene was found the most common enterotoxigenic gene. bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}1}$, bla_{KPC}, and $bla_{OXA\text{-}48}$ were found 55.8%, 46.5%, 41.2%, 18.6%, 18.6%, and 18.6%, respectively in Gram-negative strains.

Conclusion. These results is very important to highlight hygienic status of paper currencies. This can be considered as an indication that banknotes may contribute to the spread of pathogens and antimicrobial resistance. Therefore, we may need to start using alternative products instead of banknotes.

Key-words: Paper currency; Bacterial contamination; Antimicrobial resistance genes; Staphyloccoccal enterotoxins

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¿Deberíamos dejar de usar los billetes? Análisis microbiológico

RESUMEN

Objetivo. Los patógenos se pueden transmitir a los billetes debido a los hábitos antihigiénicos personales. El objetivo del estudio fue buscar los posibles patógenos en los billetes que circulan en el mercado y también observar su resistencia antibacteriana así como sus diversos factores de virulencia utilizando métodos genotípicos y fenotípicos.

Material y métodos. Se recogieron al azar un total de 150 muestras de billetes entre agosto de 2017 y marzo de 2018. Se utilizaron los sistemas VITEK para la identificación y las pruebas de sensibilidad a los antimicrobianos, respectivamente. Los genes de resistencia a los antimicrobianos (*mecA, van*, betalactamasas de espectro ampliado [BLEA] y carbapenemasas) y los genes de virulencia estafilocócica (SE, pvl y tsst -1) se determinaron mediante PCR a tiempo real.

Resultados. Se detectó la presencia de cepas de *Staphylococcus aureus*, *Staphylococcus* coagulasa negativos (SCN), *Enterococcus* spp, bacterias gramnegativas, bacterias gramnegativas no fermentativas y *Candida* spp en un 48%, 54,7%, 56%, 21,3%, 18,7% y 4% de los billetes, respectivamente. Se observó la presencia de *S. aureus* resistente a meticilina, *Enterococcus* resistentes a vancomicina y gramnegativos productores de BLEA en un 46,8%, 1,3% y 28,7%, respectivamente. Los genes Pvl, tsst-1 y SE se encontraron en un 2,8/4,9%; 1,4/1,2% y 100/87,8% de las cepas de *S. aureus*/SCN, respectivamente. El gen *sea* fue el gen enterotoxigénico más frecuente. Los genes

bla$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-1}$, *bla*$_{KPC}$, y *bla*$_{OXA-48}$ se encontraron 55,8%, 46,5%, 41,2%, 18,6%, 18,6%, y 18,6%, respectivamente en cepas gramnegativas.

Conclusión. Estos resultados son muy importantes para resaltar el estado higiénico de los billetes. De este modo, los billetes pueden contribuir a la propagación de patógenos y de la resistencia a los antimicrobianos. Por lo tanto, es posible que debamos comenzar a utilizar productos alternativos a los billetes.

Palabras clave: Papel moneda; Contaminación bacteriana; Genes de resistencia, Antimicrobianos; Enterotoxinas estafilocócicas

INTRODUCTION

The hygienic status of banknotes has been a topic of speculation since the late 1800s [1]. *In vitro* culture studies have established that microbial contamination of paper currency is widespread, and that money represents an important human-microbe interface. Microbial contamination of paper money can ocur by money counting machines, atmosphere, dust, soil, storage process, during usage or production process [2]. Contamination during use is most often caused by handwashing after the toilet or false hand washing, by saliva counting, coughing and sneezing in hands. As a result, paper money is contaminated with microorganisms from the human hand, mouth and even in the gastrointestinal tract microbiota. As a result of the exchange of these contaminated banknotes among people, microorganisms begin to spread, contributing to the spread of both antibiotic resistance and many virulence factors and they pose a risk to public health [2, 3]. Researches show that the most common microorganisms carried with paper money were enteric bacteria such as *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter* spp. and other non-fermentative Gram-negative bacilli, *Staphylococcus aureus* and other various Gram-positive cocci and various types of fungus such as *Candida* spp., *Aspergillus* spp., *Penicillium* spp. [2]. Humans are the most important source of *Staphylococcus* spp, especially *S. aureus and S. epidermidis* but also *S. hominis, S. haemolyticus, S. saprophyticus, S. capitis, S. warneri, S. simulans* and *S. cohnii*. The pathogenic capacity of these *Staphylococcus* spp. that can be easily transmitted to paper money is attributed to a combination of invasive properties, production of extracellular factors (like toxins) and antibiotic resistance. Staphylococcal toxins with superantigens characteristic include Panton-Valentin Leucocidin (PVL), toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins (ETA to ETD) and staphylococcal enterotoxins (SEs) [4]. Staphylococcal food poisoning (SFP) is caused by the ingestion of food containing SEs produced by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mainly *S. aureus*, although other CPS strains, such as *S. hyicus*, may also be enterotoxigenic [4, 5]. Recently, the enterotoxigenic potential of coagulase-negative staphylococci (CoNS) species in food poisoning has also been recognized [5-8]. There are various publications which investigated the microorganisms carried by currency banknotes [1-3, 9, 10]. However, there is limited number of studies on the dissemination of antibiotic resistance by paper money in the literature. At the same time, it has been determined that there are no studies investigating the species of staphylococci that can be carried by paper money and investigating the important virulence factors of staphylococci such as PVL, TSST-1 and SEs.

This study was planned in order to determine the microorganisms that can be transported with Turkish currency banknotes in Istanbul and to determine their role in the spread of antibiotic resistance and the potential effects of money on the spread of toxin genes by investigating the toxin genes of staphylococci.

MATERIAL AND METHODS

Bacterial isolates. A total of 150 samples of Turkish banknotes involving six denominations (5, 10, 20, 50, 100 and 200), 25 samples each, were randomly collected from hospital cafeteria, canteen of medical faculty, supermarkets near the hospital and restaurants, banks, buyers in open-air markets, and filling-stations in Istanbul, the most populated in Turkey from August 2017 to August 2018. The banknotes were obtained by using aseptic sampling method and banknotes were placed in a sterile polyethylene bag. The bag was sealed and the individual was given a replacement banknote, then all the collected samples were taken to the medical microbiology laboratory at the Medical School in Istanbul. Each banknote was placed in 10-mL of thioglicolat broth and shaken for 5–10 min on and subsequently incubated at 35-37°C for 48 hours. For isolation of bacteria, a sterile, cotton-tipped swab was introduced in the incubated thioglicolat broth and was then inoculated onto blood agar plates, Chromagar methicillin-resistant *S. aureus* (MRSA) and MacConkey agar plates and incubated at 35-37°C for 48 hours. For routine identification procedures automatized systems VITEK MS (BioMerieux, France) was used [4, 11]. For identification of fungi, a loopful of incubated nutrient broth was inoculated onto Sabouraud dextrose agar plates and incubated at 22-25°C for 48–72 hours. Identification of fungal isolates was based on growth characteristics and the lacto-phenol cotton blue reaction [4]. The isolates were stored separately in tryptic soy broth medium with 15% glycerol at –80°C for further phenotypic and genotypic analysis.

Phenotypic antibiotic susceptibility patterns of the ısolates. Phenotypic antimicrobial susceptibility testing was performed by VITEK 2 Compact (BioMerieux, France), and interpretation was done according to EUCAST-2016 guidelines [11]. MRSA isolates were defined as MRSA using a cefoxitin 30-µg disk screening test and PCR (for *mecA* gene). *S. aureus* ATCC 25923 was used as quality control [11].

Suspected isolates of *Enterococcus* spp. were screened for vancomycin resistance. The concentration of vancomycin in vancomycin screening agar was 6 mg/L. A swab which was dipped in a suspension of the isolate and then was deposited as a spot on the agar surface and it was incubated for 24 hours at 35°C. Any growth after 24 hours was interpreted as vancomy-

cin resistance [4,11]. For quality control, was used *Enterococcus faecalis* ATCC 29212 as a susceptible control and *Enterococcus faecium* ATCC 51299 as a resistant control.

Isolates of Gram-negative bacilli were inoculated on MH-agar plates. Discs containing respectively ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) and aztreonam (30 µg) disks were placed 20 mm (center to center) away from a disc containing a 20 µg amoxicillin/10 µg clavulanic acid disk before overnight incubation at 37°C. Extended-spectrum β-lactamase (ESBL) production was considered positive when the clavulanate mediated enhancement of the activity of an indicator drug produced a keyhole effect and regarded as a phenotypic confirmation of the presence of ESBL [11].

M**olecular detection.** Template DNA was prepared by a simple and rapid boiling procedure from suspension of *S. aureus* colonies [12]. DNA was collected and stored at -20°C until real-time PCR runs.

a) Molecular detection of staphyloccoccal *mecA* genes. Real-time polymerase chain reaction (PCR) was used for detection of *mecA* (table 1). As positive controls, *S. aureus* ATCC BAA-41 was used. Light Cycler 480 Probe Master kit (Roche Diagnostics GmbH, Mannheim, Germany) was used with these primers and probes on Light Cycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany) instrument according to the manufacturer's instructions [12]. Real-time PCR profile was used; denaturation step at 95°C for 10 min, followed by 45 cycles, of 10s at 95°C, 30s at 55°C, 1s at 72°C.

Molecular detection of *van* genes in *Staphylococcus* spp., *Enterococcus* spp., and ESBL genes in Gram-negative strains. Primers of *vanA, vanB, vanC1, vanC2-C3 gene*s for *Staphylococcus* spp. and *Enterococcus* spp. and beta lactamase & carbapenemase (bla_{CTX-M1}, bla_{CTX-M2}, bla_{KPC}, bla_{OXA-48}, bla_{SHV} and bla_{TEM}) genes for Gram-negative strains were provided from Integrated DNA Technologies (IDT, Coralville, IA) (table 1) [13-16]. Light Cycler 480 Sybr Green I Master kit (Roche Diagnostics GmbH, Mannheim, Germany) was used with these primers on Light Cycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany) instrument according to the manufacturer's

Table 1 **Primers used for *mecA*, van, ESBL and carbapenemase genes presence in the real-time PCR assay**

Name	Name	Oligonukleotid sequence	Ref.
mecA primers for *Staphylococcus*	MecA F	5-GGCAATATTACCGCACCTCA-3	McDonald et al, 2005 [12]
	MecA R	5-GTCTGCCACTTTCTCCTTGT-3	McDonald et al, 2005 [12]
	MecA probe	5-FAM- AGATCTTATGCAAACTTAATTGGCAAATCC-Tamra-3	McDonald et al, 2005 [12]
van gene primers for *Enterococcus* spp. and *Staphylococcus* spp.	vanA F	5-AATACTGTTTGGGGGTTGCTC-3	Khan et al, 2005 [13]
	vanA R	5-CTTTTTCCGGCTCGACTTCCT-3	Khan et al, 2005 [13]
	vanB F	5-GCGGGGAGGATGGTGCGATACAG-3	Khan et al, 2005 [13]
	vanB R	5-GGAAGATACCGTGGCTCAAAC-3	Khan et al, 2005 [13]
	vanC1 F	5-TTGACCCGCTGAAATATGAAGTAA-3	Khan et al, 2005 [13]
	vanC1 R	5-TAGAACCGTAAGCAAAAGCAGTCG-3	Khan et al, 2005 [13]
	vanC2-C3 F	5-GCATGGCAAATACGGGGAAGAT-3	Khan et al, 2005 [13]
	vanC2-C3 R	5-CATGGCAGGATAGCGGGAGTGA-3	Khan et al, 2005 [13]
ESBL and carbapenemase genes primers for Gram-negative bacilli	blaCTX-M-1	5-GCGTGATACCACTTCACCTC-3	Copur et al, 2013 [14]
		5-TGAAGTAAGTGACCAGAATC-3	
	blaCTX-M-2	5- TGATACCACCACGCCGCTC-3	Copur et al, 2013 [14]
		5-TATTGCATCAGAAACCGTGGG-3	
	blaKPC	5- CGTTCTTGTCTCTCATGGCC-3	Poirel et al, 2004 [15]
		5- CCTCGCTGTGCTTGTCATCC-3	
	blaOXA-48	5- TTGGTGGCATCGATTATCGG-3	Poirel et al, 2004 [15]
		5- GAGCACTTCTTTTGTGATGGC-3	
	blaSHV	5- ATGCGTTATATTCGCCTGTG-3	Copur et al, 2013 [14]
		5-TTAGCGTTGCCAGTGCTC-3	
	TEM	5- AGTATTCAACATTTYCGTGT-3	Copur et al, 2013 [14]
		5- TAATCAGTGAGGCACCTATCTC-3	

Table 2 **Frequency distribution [%] of microorganisms isolated from paper currencies**

Microorganisms	Paper currencies [n=25 each other]						Total [n=150]
	5£	10£	20£	50£	100£	200£	
Bacillus spp.	20	17	14	14	12	13	90 (60%)
Corynebacterium spp.	4	4	1	2	1	2	14 (9.3%)
Staphylococcus aureus	14	12	10	11	10	15	72 (48%)
Coagulase negative staphylococci (CoNS)	21	17	15	14	9	6	82 (54.7%)
Streptococcus spp.	1	1	0	0	0	0	2 (1.3%)
Micrococcus spp.	2	1	1	0	0	0	4 (2.7%)
Enterococcus spp.	8	18	12	15	11	20	84 (56%)
Neisseria spp.	1	1	1	0	0	0	3 (2%)
Escherichia coli	2	0	0	0	0	2	4 (2.7%)
Enterobacter cloacae	10	2	1	1	1	0	15 (10%)
Pantoea agglomerans	1	0	3	3	2	0	9 (6%)
Klebsiella pneumoniae	2	1	0	0	1	0	4 (2.7%)
Klebsiella oxytoca	1	1	0	0	0	0	2 (1.3%)
Pseudomonas aeruginosa	4	2	2	1	1	0	10 (6.7%)
Pseudomonas putida	2	2	0	0	1	1	6 (4%)
Acinetobacter baumannii complex	9	3	1	0	0	2	15 (10%)
Candida spp.	0	0	3	2	0	1	6 (4%)
Total	102	82	64	63	49	62	422

instructions. Real-time PCR profile was used; denaturation step at 95°C for 10 min, followed by 35 cycles of amplification; 10s at 95°C, 30s at 52°C, 1s at 72°C and melting curves; 5s at 95°C, 60s at 65°C, and 97°C cont. reading). *E. faecium* ATCC 51559, *E. faecalis* ATCC 51299, *E. gallinarum* ATCC 49573, and *E. casseliflavus* ATCC 25788 strains were used as a positive control for *vanA, vanB, vanC1,* and *vanC2-C3* genes respectively. *K. pneumoniae* ATCC 700603 and *E.coli* ATCC 25922 were also used as a control of beta lactamase and carbapenemase genes.

b) Molecular detection of SEs, pvl and tsst -1 genes. Real-time polymerase chain reaction (real-time PCR) was used for detection of specific genes to confirm their identities (such as SEs, *pvl*, and *tsst-1* gene) via the primers previously described Peck et al [17]. Light Cycler 480 Sybr Green Master kit (Roche Diagnostics GmBH, Mannheim, Germany) was used with these primers on Light Cycler 480 II (Roche Diagnostics GmBH, Mannheim, Germany) instrument according to the manufacturer's instructions. 0.5 uM primers were added in reactions of final concentrations. Real-time PCR profile was used; denaturation step at 95°C for 10 min, followed by 40 cycles, of 10s at 95°C, 30s at 55°C, 1s at 72°C and melting curves; 5s at 95°C, 60s at 65°C, and 97°C cont. reading). As positive controls, *S. aureus* ATCC 13565 (*sea, sej*), *S. aureus* ATCC 14458 (*seb*), *S. aureus* ATCC 19095 (*sec, seh*), *S. aureus* ATCC 23235 (*sed, seg, sei*), *S. aureus* ATCC 27664 (*see*), *S. aureus* ATCC 25923 *(pvl*), *S. aureus* ATCC 51650 (*tsst-1*) were used. As a nontoxigenic control *S. aureus* ATCC 6538 was used.

RESULTS

Of the 150 samples of Turkish currency banknotes on which bacteriological analysis was conducted, 81% were found to be contaminated with several microbial species. The spectrum of microbial species were detected at rates of; *S. aureus* 48% (46.8% MRSA and 1.2% MSSA), CoNS 54.7%, *Enterococcus* spp. 56%, enteric bacteria 21.3%, non-fermentative Gram-negative bacteria 18.7% and *Candida* spp. 4%. A wide distribution of pathogens occurred from the different points included (table 2). The highest microbial contamination was obtained in the Turkish currency banknotes from the hospital cafeteria, followed by the cafeteria of medical faculty students. Others were with order supermarkets and restau-

Table 3 **Identification of ESBL and carbapenemase genes in Gram-negative bacilli**

Bacteria (number of isolates/EBSL positive)					
E. coli (n=4/3)	*K. pneumoniae* (n=4/4)	*E. cloacae* (n=15/10)	*P. agglomerans* (n= 9/4)	*A. baumannii* (n=15/12)	*P. aeruginosa* (n=10/7)
TEM + KPC + SHV (1 strain)	CTX-M-1 + OXA 48 (1 strain)	TEM (1 strain)	SHV (1 strain)	CTX-M-1 + OXA-48 (1 strain)	CTX-M-1 + KPC (1 strain)
CTX-M-2 + TEM + SHV (1 strain)	CTX-M-2 + KPC (1 strain)	CTX-M-2 (1 strain)	CTX-M-2 + TEM + SHV (2 strains)	CTX-M-2 + OXA-48 (1 strain)	CTX-M-1 + TEM + SHV (1 strain)
CTX-M-2 + TEM + OXA-48 + SHV (1 strain)	TEM + OXA-48 (1 strain)	CTX-M-2 + TEM (2 strains)	CTX-M-1 + CTX-M-2 + TEM + SHV (1 strain)	CTX-M-2 + TEM (3 strains)	CTX-M-2 (1 strain)
	TEM + OXA-48 + SHV (1 strain)	CTX-M-1 + TEM + SHV (1 strain)		KPC (2 strains)	CTX-M-2 + TEM (2 strains)
		TEM + SHV (2 strains)		OXA-48 + SHV (2 strain)	TEM + OXA-48 (1 strain)
		CTX-M-2 + TEM + SHV (2 strains)		SHV (3 strains)	KPC (1 strain)
		CTX-M-1 + CTX-M-2 + SHV (1 strain)			

rants around the hospital, banks, buyers in open-air markets and filling-stations. In the Turkish currency banknotes, the most intensive bacterial contamination was found in 5£, followed by 10£, 20£, 50£ and 100£, respectively. When looking at 200£banknotes, the contamination rate was found to be higher than 100£. The species of *Staphylococcus* spp. 154 produced in the highest proportion were *S. aureus* 48% and CoNS 54.7%. The distribution of CoNS were *S. epidermidis* 46.7%, *S. haemolyticus* 20%, *S. hominis* 12.2%, *S. capitis* 11%, *S. warneri* 4.9%, *S. lugdunensis* 3.7%, *S. caprae* 2.4% and *S. saprophyticus* 2.4%. The *mecA* gene was observed in 90.3% of *S. aureus* and in 73% of CoNS isolates. When the antibiotic resistance of *Staphylococcus* spp. were examined; the resistance rates in MRSA strains were erythromycin 66.7%, clindamycin 22.2%, gentamicin 16.7%, trimethoprim+sulfamethoxazole (SXT) 16.7%; In *S. epidermidis*, erythromycin 34.3%, clindamycin 17.2%, gentamicin 5.9%, ciprofloxacin 5.9% and SXT 5.9%; in *S. haemolyticus* erythromycin 72.2%, clindamycin 44.4%, tigecycline 38.9%, ciprofloxacin 38.9% and linezolid 38.9%; in *S. hominis* erythromycin 16.2% and SXT 16.2%; in *S. capitis* gentamicin 20%. None of the staphylococci strains were found to have quinupristin/dalfopristin and vancomycin resistance. The rate of multi-drug resistance (resistance to more than three antibiotics-MDR) was found as 40.3%.

The second most frequently isolated 84 *Enterococcus* spp. (56.7%) was the distribution of bacteria in the species *E. faecium* 35 (41.7%), *E. faecalis* 8 (9.5%), *E. casseliflavus* 21 (25%) and other *Enterococcus* spp. 10 (11.9%), respectively. Vancomycin resistance was determined by both phenotypic and genotypic methods in two origins, one *E. faecium* and one *E. casseliflavus* (2.4%). The resistance gene was *vanA*. Other vancomycin resistance genes were not detected. Enteric bacteria isolated from banknotes were 21.3%. *Enterobacter cloacae* was the first line of enteric bacteria with 46.9%. The others were *Pantoea agglomerans* 28.2%, *E. coli* 12.5%, *K. pneumoniae* and *K. oxytoca* 12.5%, respectively. When the antimicrobial resistance in enteric bacteries was examined ampicillin was found to be with the highest resistance rate as 81%. Resistance rates to other antibiotics were determined as follows: ceftazidime 75%, cefuroxime and cefuroxime + axetil combination of 65.6% cefoxitine 62.5%, cefepime 78%, ceftriaxone 9.4%, ertapenem, meropenem, imipenem 12.5%, amikacin 25%, gentamicin 22%, ciprofloxacin 40.6%, tigecycline 3% trimethoprim-sulfamethoxazole 25%, colistin 6.3%. MDR in enteric bacteria was 40.6%. ESBL enzyme genes were found to be 66.7% in enteric bacteria (table 3). Non-fermentative Gram-negative rods isolated from banknotes were 18.7%. Among the non-fermentative bacteria, *Acinetobacter baumannii* complex ranked first with 53.6%. The others *were P. aeruginosa* 35.7%, *P. putida* 10.7% and *P. stutzeri* 7.2% respectively. Antimicrobial resistance rates of *Pseudomonas* spp. were as piperacillin 50%, piperacillin+ tazobactam 40%, ceftazidime 40%, ceftriaxone 30%, imipenem 10%, amikacin 20% and ciprofloxacin 30%. Antimicrobial resistance rates of *Acinetobacter baumannii* complex were as piperacillin 53.3%, piperacillin+ tazobactam 40%, ceftazidime 66.7%, ceftriaxone 33%, imipenem 26.7%, amikacin 33% ve ciprofloxacin 46.7%. MDR was 60% in *P. aeruginosa* and 76% in *A. baumannii* complex. ESBL enzyme genes were found to be 65.6% in enteric bacteria and 76% in non fermentative Gram-negative bacteria. The distribution by species was *E. coli* 75%, *K. pneumoniae* 100%, *E. cloacae* 66.7%, *P. agglomerans* 44.4%, *P. aeruginosa 70%* and *A. bau-*

Table 4 Status of *mecA* and toxin genes of 154 *Staphylococcus* spp. isolates

	Number of positive isolates							
Bacterial isolates (n)	*mecA*	Pvl	Tsst-1	SEs	Only one toxin gene	Multiple toxin gene	Toxigenic	Non-toxigenic
S. aureus (72)	65	2	1	72	1	71	72	0
CoNS (82)	60	4	1	74	3	71	74	8
S. epidermidis (35)	29	1	0	35	1	34	35	0
S. haemolyticus (17)	8	1	0	12	1	11	12	5
S. hominis (10)	9	1	0	10	1	9	10	0
S. capitis (9)	7	0	0	9	0	9	9	0
S. warneri (4)	3	1	1	4	0	4	4	0
S. lugdunensis (3)	2	0	0	2	0	2	2	1
S. caprae (2)	1	0	0	1	0	1	1	1
S. saprophyticus (2)	1	0	0	1	0	1	1	1
Total (154)	125	6	2	146	4	142	146	8

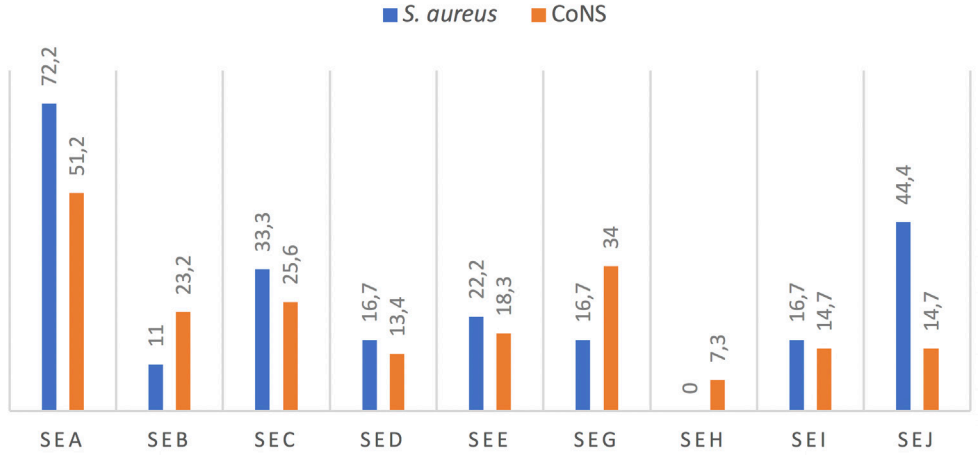

Figure 1 Distribution of SEs genes in *S. aureus* and CoNS

mannii 80% (table 3). CTX-M type ESBL enzyme genes were found to be 43.8% in enteric bacteria and 40% in non fermentative Gram-negative bacteria. The distribution by species was *E. coli* 50%, *K. pneumoniae* 50%, *E. cloacae* 46.7%, *P. agglomerans* 22.2%, *P. aeruginosa* 50% and *A. baumannii* 33.3%. In our study, bla_{KPC} was found as 6.2 % in enteric bacteria and as 12 % in non-fermentative bacteria. The distribution by species was *E. coli* 25%, *K. pneumoniae*, 25%, *P. aeruginosa* 20% and *A. baumannii* 13.3%. OXA-48 enzyme genes were found to be 12.5 % in enteric bacteria and 20% in non-fermentative Gram-negative bacteria. The distribution by species was *E. coli* 25%, *K. pneumoniae* 75%, *P. aeruginosa* 10% and *A. baumannii* 26.7%. The availability of toxin genes were 100% in *S. aureus*, 100% in *S. epidermidis*, 70.6% in *S. haemolyticus*, 66.7% in *S. lugdunensis*, 50% in *S. caprae and* 50% in *S. saprophyticus*. The distribution of toxin genes were *pvl* 2.8%, *tsst-1* 1.4% and SEs 100% in *S. aureus, pvl* 4.9%, *tsst-1* 1.2% and SEs 87.8% in CoNS (table 4). The distribution of SEs genes in *S. aureus* were as *sea* 72.2%, *seb* 11%, *sec* 33.3%, *sed* 16.7%, *see* 22.2%, *seg* 16.7%, *sei* 16.7% and *sej* 44.4%, the *seh* gene was not found. The distribution of SEs genes in CoNS were as *sea* 51.2%, *seb* 23.2%, *sec* 25.6%, *sed* 13.4%, *see* 18.3%, *seg* 34%, *seh* 7.3%, *sei* 14.7% and *sej* 14.7% (figure 1). Comparing with that of CoNS, the *sea* gene was found statistically significantly high in *S. aureus* strains ($p<0.05$) and comparing with that of *S. aureus* strains, the *seb*, *seg and seh* genes were found statistically significantly high in CoNS strains.

DISCUSSION

Paper currencies are objects capable of absorbing, harboring and transmitting infectious microorganisms [2]. Researches show that the microbial load on banknotes varies according to the banknotes, seasons, stored under varying environmental conditions, the age of banknotes, the local community microbiota, the general hygiene level, and the general hygienic conditions [3, 9, 10]. The amount of bacterial contamination on currency varies widely between countries. Previous studies have revealed that 70-97% of banknotes harbor various bacteria and viruses on the surface in different nations such as the United States, Mexico, China, India, Saudi Arabia, Sudan, Pakistan, Brasil etc, [9, 10, 18, 19, 20]. In our study, Turkish currency banknotes on which bacteriological analysis was conducted, 81% were found to be contaminated with several microbial species. Our results show that similar results were obtained in previous studies. Numerous studies have shown that cotton-based banknotes have more microbial loads than polymer-based ones [3, 10].

Vriesekoop et al. reported that comparison of cotton-based banknotes of countries such as China, Ireland, The Netharlands, Nigeria, United Kingdom and the United States, as well as the polymer-based banknotes of countries such as Australia and New Zealand. They found that cotton-based banknotes had much more bacterial loading than polymer-based banknotes [3]. The bacterial load evaluated as 81% in Turkish banknotes can be explained by the fact that they are based on cotton. Some studies showed that, the longer the paper currencies remain in circulation, the more chance there is for them to become contaminated, and lower-denomination notes receive the most handling because they are exchanged more frequently [2, 3]. According to our results also showed that health centers and health center workers and people who stay here play an important role contributing to the bacterial contamination. Many previous studies also claimed similar results [2, 3, 9, 10]. Many bacteria have been isolated from banknotes in studies from Turkey, China, Philippines, India, Saudi Arabia, Mexico, New Zealand, Australia, Canada, USA and Europe. It was also reported that *S. aureus, E. coli, Klebsiella* spp. and *Enterobacter* spp. were identified from these countries' banknotes [9, 10]. *Staphylococcus* spp. present in the nose often contaminate hands, fingers, faces, and nasal carriers which can easily become skin carriers [4]. In general, there was no obvious difference in survival between multiresistant and susceptible *S. aureus* strains. *S. aureus* (including MRSA) survive for 7 day -7 months on dry surfaces [21]. In our study, *Staphylococcus* spp. were the most isolated bacteria. Previous studies have also determined that there is a high number of *Staphylococcus* spp. on banknote, however, most studies did not identify *Staphylococcus* spp. Our study was the first research to identified *Staphylococcus species* unlike other researches on this topic. Methicillin resistance is an important consideration in all *Staphylococcus* spp., especially *S. aureus.* Global transmission of MRSA has been the subject of many studies [3, 22]. In recent study, it was determined that the rate of methicillin resistant *S. aureus* was 90.3% and methicillin resistant in CoNS is 73.2%. The highest antibiotic resistance in staphylococcus was erythromycin (72.2%), and clindamycin (44.4%) resistance in *S. haemolyticus*; gentamicin resistance (20%) in *S. capitis*. Tigecycline (38.9%), ciprofloxacin (38.9%) and linezolid (33.3%) resistance were found only in *S. haemolyticus* strains. None of the staphylococci strains had resistance to quinupristin/dalfopristin and vancomycin. The rate of MDR was found as 40.3%. Recently, many published studies reported that *E. faecium* infections are increasing worldwide [4]. In our country, the rates of *E. faecium* and *E. faecalis* were determined to be 15 - 50% and 52 - 85%, respectively [23, 24]. In previous researches, *Enterococcus* spp., which can be found without losing their vitality for 4 months in inanimate environments [21].

Many Gram-negative species, such as *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Serratia marcescens*, or *Shigella* spp. can survive on inanimate surfaces even for months. Overall, Gram-negative bacteria have been described to persist longer than Gram-positive bacteria [3, 21]. Humid conditions improved persistence for most types of bacteria, such as *Salmonella typhimurium, P. aeruginosa, E. coli* or other relevant pathogens [2, 9, 21, 25]. In previous studies, reported that *Enterobacteraceae* members are 13%-55.5% range of the paper currencies and the most frequently isolated enteric bacteria was *E. coli* (19.4-48.14%) [2, 3, 9]. Antimicrobial resistance is a global phenomenon that has resulted in high morbidity and mortality as a result of treatment failures and increased health care costs. Research has shown that contaminated fomites in general and paper currency in particular, plays a key role in the spread of bacterial infections with antimicrobial resistance [2, 3, 25]. Heshiki et al. [22] in a metagenomic study showed that the antimicrobial resistance genes on banknotes were significantly higher (4.86 times more) than environmental samples such as water, air, soil and dust.

Emergence of glycopeptide resistance causes more severe prognosis, higher mortality, and recurrence in enterococcal infections. The most common type of enterococcal vancomycin resistance is high-level resistance associated with acquisition of the *vanA* and *vanB genes*, typically observed in *E. faecium* and *E. faecalis* isolates [4]. Conversely, the *vanC* genotype is associated with constitutive low-level vancomycin resistance and is intrinsic to *E. gallinarum* and *E. casseliflavus* [4]. In our study, vancomycin resistance was determined by both phenotypic and genotypic methods in two isolates (2.4%), these were one *E. faecium* and one *E. casseliflavus.* The resistance genes were *vanA.* Other vancomycin resistance genes were not detected.

Resistance mediated by ESBLs includes all penicillins, cephalosporins (including third-generation cephalosporins) and aztreonam. Since plasmid-mediated ESBLs were first detected in a *K. pneumoniae* isolate in 1983 in Germany [26]. A new non-TEM non-SHV ESBL was isolated in Germany, in 1989, in a strain of *E. coli* called CTX-M because of its preferential activity on cefotaxime rather than ceftazidime [27]. Over the past 20 years, some *Enterobacteriaceae* mainly *E. coli, K. pneu-*

moniae, and *Proteus mirabilis* have demonstrated acquisition of plasmids secreting ESBL [28]. In our study, the rates of ESBL enzyme genes were found to be high as 65.6% in enteric bacteria and as 76% in non-fermentative Gram-negative bacteria. CTX-M type ESBL enzyme genes were found to be 43.8% in enteric bacteria and 40% in non-fermentative Gram-negative bacteria. Carbapenemases in *Enterobacteriaceae* are mainly found in *K. pneumoniae*, and to a much lesser extent in *E. coli* and other enterobacterial species, with a higher prevalence in southern Europe and Asia than in other parts of the World [28]. The first OXA-48 carbapenemase was identified in 2001 from a K. pneumoniae isolate obtained from a urine specimen collected in Istanbul, Turkey [15]. Shortly thereafter there was an outbreak of OXA-48 producing *K. pneumoniae* isolates reported in Istanbul in 2006 [29]. In our study, OXA-48 enzyme genes were found to be 12.5% in enteric bacteria and 20% in non fermentative Gram-negative bacteria. *Staphylococcus* spp. are also capable of producing "distant" diseases, which are mediated by the secretion of toxins and these toxins can be produced directly by bacteria that colonize the skin or mucosa or *indirectly* by microorganisms that colonize food, beverages and fomites [4, 30]. Bacteriological studies about banknotes, have included no analysis of the toxin genes (*pvl, tsst-1* and SEs). 95.4% of *Staphylococcus* spp. that are analyzed from our study were determined to possess toxin genomes. The distribution of these toxin genomes was as follows: 3.9% *pvl,* 1.3% *tsst-1* and 98.4% SEs. There was no toxin genomes in the rest of the *Staphylococcus* spp. (5.2%). PVL is a cytotoxin that causes tissue necrosis and leukocyte destruction. This linkage to virulent strains suggests its capability of causing deadly infections in healthy people [4]. Toxic shock syndrome (TSS) is a life-threatening illness characterized by high fever, erythematous rash with subsequent desquamation of the skin, shock, and multiple organ involvement [4, 31]. In our study, it was possible to detect 1.3% of tsst-1 genomes from our isolated banknotes.

Six enterotoxins serotypes (*sea* to *see* and *seh*) have been involved in most of the *Staphylococcus* poisoning outbreaks worldwide [31]. In our study, it was indicated that 94.8% of *Staphylococcus* spp. have SEs genomes. *S. aureus* and CoNS strains can encode more than one enterotoxin gene simultaneously; over 50% of the isolates assessed showed this property [8]. All *S. aureus* strains were carried at least one SEs gene and the combination *sea+sei, sea+sec+sei, sea+sed+sej* was the most frequent. CoNS strains were positive SEs genome 90.2% and the combination *sea+sej, sea+sec+sej, sea+seg+sej, sea+sed+seg+sei* was the most frequent. *sea* is one of the most frequently observed enterotoxins, although the literature shows highly variable results in the prevalence of *S. aureus* enterotoxin genes, depending on the kind of food and the biovar investigated [4, 31]. Compared to CoNS strains, *sea* genes were statistically significantly higher in *S. aureus* strains ($p<0.05$). Compared to *S. aureus* strains, *seb, seg* and *seh* genes were statistically significantly high in CoNS strains ($p<0.05$). On the other hand, the *seh* gene was detected at a rate of 7.3% in CoNS strains, although there was no in the *S. aureus* strains.

Several factors in the spread of pathogen and potential pathogenic bacteria, as well as antimicrobial resistance and virulance genes such as SEs, community and hospital enviroments, animal products and the environmental compartment are important. Results of this study in terms of demonstrating that paper curriencies or banknotes circulating in society can potentially mediate the transport of microorganisms among people and poses a risk to public health and it is also very important to highlight the need for proper hygienic practices for maximally reducing the spread of disease-causing pathogens. This can be considered as an indication that banknotes may contribute to the spread of pathogens and antimicrobial resistance. In this study, it was aimed to pay attention to hand hygiene for reducing the microbial load on the currencies and the necessity of producing these banknotes with maintain less bacteria such as plastic etc. instead of cotton. In addition, our study has been the first research to identified staphylococcus species and its virulance genes unlike other researches on this topic.

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None to declare

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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