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RESEARCH ARTICLE

COMMON BACTERIA ISOLATES FROM SUDANESE BANKNOTES CIRCULATING BETWEEN HANDLERS IN KHARTOUM STATE, SUDAN.

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Key words:-

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Banknotes handlers, Sudan.

Abstract

Background: Banknotes are commonly and routinely circulating among individuals, and bacteria contaminated sudanese banknotes can spread and transmitted directly, through hand-to-hand contact, or indirectly, via food or other inanimate objects. This study was designed to assess the bacterial contamination of Sudanese banknotes and banknotes handlers hygiene in Khartoum state, Sudan.

Methods: Fifty samples of Sudanese banknotes were collected randomly from handlers through (March - April / 2015). These samples were microbiologically tested for quantify and identification of bacteria contaminated of Sudanese banknotes by using biochemical tests and (SPSS) system to analyzed the study questionnaires.

Results: The mean of total viable count (CFU/cm²) of bacteria contaminated of banknotes between (4.5 – 8.3) x 10⁵ CFU/cm² , the study showed 12 different types of bacteria identified from Sudanese banknotes with different percentages and From statistically analyzed of 50 questionnaires there were; 76% of handlers had knowledge about contaminated banknotes as being a source of some diseases; 94% educated handlers at different levels; 64% of the handlers washed hands after using a toilet and before dealing with banknotes again.

Conclusions: Sudanese banknotes were contaminated with different bacteria. Unhygienically handled between handlers . The contamination rate was higher in lower denominations than in higher ones.

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Introduction:-

Banknote is widely exchanged for goods and services. It is offer ample surface area to harbor of microorganisms, and the hygienic status of currency has been a scourge to some for over a century, not many authors - even in Sudan - have raised the concern that banknotes could serve as vectors for the transmission of diseases (Pittet, *et al.*, 2006). It is commonly and routinely circulating among individuals, and microbes can spread on the surface of paper currency. Paper currency is made of a rugged mix of (75% cotton and 25% linen) mostly, and offers surface area for bacteria and microorganisms to reside on both sides.

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Polymer-based banknotes presented lower bacterial counts than cotton-based banknotes. It is possible that the fibrous surfaces of cotton-based banknotes provide a good surface for bacterial attachment (Vrieskoop, *et al.*, 2010).

Health care associated infections are one of the most serious patient safety issues in healthcare today. Most pathogens are able to survive on surfaces and these surfaces can act as sources of pathogen transmission if no disinfection is performed, and banknotes are handled by persons of varying health and hygienic standards, and are stored under varying environmental and personal hygienic conditions.

However, only few data are available about the types of patient care activities that are able to transmit the patient flora to healthcare workers' hands (Pittet, *et al.*, 2006).

The possibility of banknote contamination with bacteria has also been observed among food handlers. An assessment of the public health risk associated with the simultaneous handling of food and money in the food industry in Australia (Brady and Kelly, 2000).

An investigation that was reported in 1997 and that involved swabbing and culturing from various coins and banknotes collected randomly from doctors, laboratory staff and other employees at a New York hospital resulted in the recovery of many pathogenic microorganisms (FSA, 2000).

In reality, banknotes contaminated with various bacteria, after all, we hardly expect them to be sterile.

All studies that have come across investigating bacterial contamination on money (banknotes or coins) have found a significant proportion (53% - 100%) to be contaminated (Uneke and Ogbu, 2007).

The amount of bacterial contamination on banknotes varies widely between countries. As a result, 88% of the banknotes tested in Jeddah, Saudi Arabia were contaminated with a variety of microorganisms, and 94% of US\$1 bills had bacterial contamination (Al-Gamdi, *et al.*, 2011).

Approximately 80% of the banknotes tested in Bangladesh and 89% of the banknotes tested in Nigeria had bacterial contamination, whereas in Ghana, 100% of the banknotes tested were found to be contaminated with one or more bacterial species (Tagoe, *et al.*, 2010).

Money has often been thought of as dirty. In fact, the association of money with disease transmission has long been established. Even from a historical perspective there have been reports that villagers believed money was somehow responsible for plague epidemics in England (Gabriel and Coffey, 2013).

Also dirty/damaged banknotes (indication of frequent exchange) has been shown to be significantly more contaminated than clean and mint condition banknotes, and low denomination were more likely to be contaminated than higher denomination of banknotes (probably reflecting frequency of use and socio-economic factors) (Uneke and Ogbu, 2007).

The type of bacteria found on money (Banknotes or Coins) includes *E. coli*, *Vibrio* spp, *Klebsiella* spp, including

K. pneumonia - *Serratia* spp, *Enterobacter* sp, *Salmonella* spp, *Acinetobacter* spp, *Enterococcus* spp., *Staphylococcus* including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Proteus* spp, *Bacillus* spp, *Pseudomonas* spp, including *P.aeruginosa*, *Shigella* spp, *Corynebacterium*, *Lactobacillus* spp, *Burkholderia cepacia*, *Micrococcus* spp, and *Alcaligenes*.

It is clear that some of these bacteria are common environmental bacteria considered non-pathogenic.

However, many are either potentially pathogenic or common human pathogens. For example, *K. pneumonia* is a virulent organism and may cause both community and hospital-acquired infections. Even those organisms not commonly associated with disease in healthy hosts can cause clinically significant infections in immunocompromised and hospitalized patients. These include even the natural inhabitants of the human skin such as *Staphylococcus* spp (Bhalakia, 2005).

Analyzed banknotes handled by people who were also food handlers for the presence and levels of microorganisms, the presence of Staphylococci on the banknotes surface was confirmed, these related human occupational activities, especially those involving simultaneous banknotes handling, could introduce the risk of cross-contamination to foods (FSA, 2000).

An aspect of food service that frequently causes comment, particularly among enlightened consumers, is the way a food handler prepares the food, takes banknotes for the purchase, returns change to the customer, and then prepares food for the next customer. This pattern is most noticeable in sandwich bar operations, but is by no means restricted to outlets of that type, but in this example anything that gets on hands can get on banknote.

The climatic and environmental conditions favor the thriving of many pathogenic microorganisms and in the face of underdevelopment inadequate water and sanitation, crowded living conditions, lack of access to health care, low levels of education, a greater proportion of the populace, particularly the poor, become highly susceptible for infection and diseases (Gwatkin and Guillot, 2000).

Risk of infection is increased several fold when objects that change hands at a high frequency, such as banknotes, are contaminated with microbes.

The risk is by no means restricted to residents of the country, it might even be greater for expatriates, tourists, and visitors from other countries, who may not be immune to the pathogens in Sudan, poor-currency-handling culture is wide spread, and there is indiscriminate abuse of currency notes. A great majority of the populace does not carry money in wallets, and squeezing of banknotes is a common occurrence. Women, especially among the unenlightened, often place banknote under their brassier, while men place theirs in the socks.

The banknotes are assessed for criteria such as soiling, tears and ink wear. Each denomination within one country will have a different circulation lifetime, the highest denominations last considerably longer than the lowest denominations. As there is a multitude of influential factors, it is very difficult to compare banknotes life time results in different countries, the life time factor for banknotes are increase depending on the denomination (Louisenthal, 2005).

Methodology:-

Samples collection:-

Fifty samples of five Sudanese banknotes denominations, were collected randomly from workers, banks accountants, fruits and vegetables sellers, students, and the transport ticket collectors in the Khartoum State, every denomination was kept in an alcohol sterilized tube and taken directly to the microbiology laboratory of the Faculty of Public Health (UofK) for microbiological testing.

Sterilization of Materials:-

Glass wares were sterilized at 121°C for 15 minutes. Work surfaces were cleaned and sterilized by swabbing with 95% ethanol. 10 milliliter of normal saline was dispensed into McCartney bottles and then sterilized in a autoclave.

Preparation of Media:-

The media MacConkey agar, Manitol salt agar, Blood agar, Plate count agar, Nutrient agar, and Sabouraud dextrose agar were prepared and used according to the manufacture's specification.

Culturing process:-

All samples of Sudanese banknotes were analyze in a sterile atmosphere. The culturing of media followed the manufacturer's instructions.

The agar was mixed with the appropriate amount of normal saline and autoclaved at 121°C for 15 minutes, then which it was cooled until and poured aseptically into plastic Petri dishes, and allowed to cool until it is solid. Solid agar was inoculated and incubated for 24 hours, after which possible growth were checked for.

Pure colonies of isolated bacteria were identified and characterized using standard microbiological techniques.

Miles and Misra method:-

The miles and misra method (or surface viable count) is a technique in microbiology to determine the total viable count (CFU) in a bacterial suspension or homogenate. It has the advantage of being economical with agar media,

lines were drowned on the bottom of an agar plate, divided it into 4 sectors. An inoculum of 0.02 ml from each fold dilution was pipetted as a drop on the agar in each sector. The inoculated were allowed to dry and the plates were incubated at 37°C for 24 - 48 hours, then calculated of CFU/ml in the original sample.

Microbiological Tests:-

Nine milliliters of normal saline (0.8 NaCl) was dispensed into test tubes in 4 folds and then labeled sequentially from 10^{-1} to 10^{-4} .

The test tubes were plugged with cotton wool and sterilized by autoclaved at 121°C for 15 minutes. On cooled, the samples of banknotes were dissolved in flasks every one contained normal saline depending on the distance of the two surfaces of banknotes (2 SDG in 95 milliliters of normal saline, 5 SDG in 105 milliliters of normal saline, 10 SDG in 108 milliliters of normal saline, 20 SDG in 120 milliliters of normal saline, and 50 SDG in 123 milliliters of normal saline), and then mixed by shaking thoroughly and appropriately. Serial dilution method was used for total viable count on Plate account agar. Serial dilutions were carried out using sterile syringes to pipette 1ml from the mixed sample into the test tube labeled 10^{-1} and then shaken thoroughly. 1ml from 10^{-1} was pipette into the next labeled test tube 10^{-2} and then mixed, 1ml was pipette again from 10^{-2} into 10^{-3} labeled test tube and this was done sequentially to 10^{-4} . All cultures for bacteria were incubated at 37°C for 24 hours.

Gram stain:-

Procedure of Gram stain:-

Thin smear was made by emulsifying a little portion of organism picked from grown colonies of 18-24 hours old pure culture into a drop of sterile distilled water on a grease free slide. The smear was allowed to air-dry and then heat-fixed by passing it slightly over flame. The slide was carefully placed on the staining rack, and flooded with the primary stain (crystal violet) for 30-60 seconds. Gram's iodine was added (mordant) for 30 seconds. The smear was gently rinsed with tap water. Alcohol (70% ethanol) was applied to serve as a decoloriser for 10-30 seconds; it was then rinsed with tap water again and allowed to dry. The smear was examined under the microscope using x100 oil.

MacConkey agar:-

MacConkey agar was used for isolation of Gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting Gram-negative bacteria. It has also become common to use the media to differentiate bacteria by their abilities to ferment sugars other than lactose.

Blood Agar:

Blood Agar was used to grow of fastidious organisms and to differentiate bacteria based on their hemolytic properties.

Biochemical tests:-

These tests that were carried out to further identify and classify isolate, this included the following; indole test, citrate-utilization test, sugar fermentation test, kligler agar test, voges proskauer agar test, urease test, and triple sugar iron agar test.

Oxidase test:-

The oxidase test was used to assist in the identification of oxidase producing organisms. A piece of filter paper was soaked with few drops of oxidase reagent Tetra methyl phenyl enediamine dihydrochloride.

A colony of that test organism was then smeared on soaked filter paper. If organism was oxidase producing organism, the phenyl enediamine in the reagent was oxidized to deep purple color.

The change to deep purple color within 10 seconds indicates positive result. It is used to separate enteric from *pseudomonads*.

Catalase test:-

The catalase test was used to demonstrate the presence of catalase an enzyme characterized with the release of oxygen from hydrogen peroxide H_2O_2 . Pure culture of the test organism was required for the test, a drop of 3% H_2O_2 solution was added to the microscope slide, a loop of the organism was touched to the drop of H_2O_2 . Foaming or bubbling indicates a positive result.

Coagulase test:-

Coagulase is an enzyme that clots blood plasma. This test was performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus*.

Indole test:-

This test differentiates coliforms by the ability to produce the amino acid, tryptophan. 5ml of prepared medium was transferred into McCartney bottle and autoclaved at 121°C for 15 minutes after which it was inoculated with the test organism. This was incubated at 37°C for 24 hours after which 2-3 drops of Kovacs reagent was added to the cultured organism. A pink to red color indicates a positive result while yellow color indicates negative result.

MR-VP (Methyl red-Voges Proskauer):-

This test was used to determine two things. The MR portion (methyl red) is used to determine if glucose can be converted to acidic products like lactate, acetate, and formate. The VP portion is used to determine if glucose can be converted to acetone.

These tests were performed by inoculating a single tube of MR-VP media with a transfer loop and then allowed the culture to grow for 3-5 days. After the culture grew, about half of the culture was transferred to a clean tube. One tube of culture was used to conduct the MR test, the second tube served as the VP test.

MR (methyl red) test:-

Methyl red was added to the MR tube. A red color indicates a positive result (glucose can be converted into acidic end products such as lactate, acetate, and formate). A yellow color indicates a negative result; glucose was converted into neutral end products.

VP (Voges Proskauer) test:-

First alpha-naphthol (also called Barritt's reagent A) and then potassium hydroxide (also called Barritt's reagent B) were added to the VP tube. The culture should be allowed to sit for about 15 minutes for color development to occur. If action was produced then the culture turns a red color (positive result); if action was not produced then the culture appears yellowish to copper in color (a negative result).

Citrate Utilization:-

This test for the ability of bacteria to convert citrate (an intermediate of the Krebs's cycle) into oxaloacetate (another intermediate of the Krebs's cycle).

In this media, citrate was the only carbon source available to the bacteria. If it can't use citrate then it was not grown. If it can use citrate, then the bacteria was grown and the media was turned a bright blue as a result of an increase in the pH of the media.

Urea test:-

This test was used to detect the enzyme urease, which breaks down urea into ammonia. Ammonia was a base and thus raised the pH of the media, if it was present. This change in pH was indicated by a pH indicator called phenol red which was present in the media. A color change from yellow to bright pinkish-red it was positive; lack of color change was a negative result.

Triple sugar Iron (TSI) and Hydrogen sulfide production (H₂S):-

Looks at fermentation of glucose, lactose, and sucrose and checks if hydrogen sulfide is produced in the process. Basically a pH indicator will change the color of the media in response to fermentation where that color change occurs in the tube will indicate what sugar or sugars were fermented. The presence of a black color indicates that H₂S was produced. In this media, H₂S reacts with the ferrous sulfate in the media to make ferrous sulfide, which is black. To inoculate, use a needle to stab agar and then use a loop to streak the top slanted region. In addition to TSI media, SIM media can be used to determine if H₂S is produced.

A black color in the SIM medium following inoculation and incubation indicates that H₂S is made by the bacteria.

Starch hydrolysis:-

This test was used to detect the enzyme amylase, which breaks down starch. After incubated the plate was treated with Gram's iodine. If starch was hydrolyzed (broken down) then there was a reddish color or a clear zone around

the bacterial growth; if it was hydrolyzed then there was a black/blue area indicated to presence of starch. Simply used inoculated loop to spread bacteria onto plate surface. After the bacteria have grown, I have added a few drops of Gram's iodine to the plate and looked for the color immediately after added the iodine.

Fermentation test:-

The ability of the isolates to utilize certain sugars as the only source of energy was tested. Peptone medium was prepared according to the manufacturer's specification and few drops of phenol red were added. 1% solution (1 gm in 100 ml distilled water) of the sugar under test was also prepared separately in McCartney bottles. 1 ml of the 1% solution was added to 9ml of phenol red peptone medium in test tubes and mixed gently.

The test tubes were covered with cotton wool wrapped in aluminum foil and sterilized at 121°C for 15minute. On cooled, the test tube were inoculated with the bacteria isolated and incubated at 37°C for 48 hours. Color changed from red to yellow indicated acid production.

Additional Non-Biochemical Tests:-

Motility test:-

This test was used to check for the ability of bacteria to migrate away from a line of inoculation, the bacterial sample was inoculated into SIM or motility media using a needle. The media was started in as straight a line as possible and with drawn the needle very carefully.

After incubation the sample for 24-48 hours. Migration away from the line of inoculation the microorganism was motile (positive test). Lack of migration away from the line of inoculation indicated a lack of motility (negative test result), (Cowen, 1981).

Results:-

Results of microbiological tests:-

Table (1):- The total viable count for control samples of Sudanese banknotes. All samples without contamination in (table1):

Value of Banknote	CFU/cm ² of control samples
2 SDG	0.0
5 SDG	0.0
10 SDG	0.0
20 SDG	0.0
50 SDG	0.0

Table (2):- The mean of total viable count of Sudanese banknotes. All banknotes were contaminated, (the denomination 2 SDG showed the highest mean of contamination in table (1)):

Value of Banknote	Mean of total viable count CFU/cm ²
2 SDG	8.3 x 10 ⁵
5 SDG	7.2 x 10 ⁵
10 SDG	6.0 x 10 ⁵
20 SDG	4.7 x 10 ⁵
50 SDG	4.5 x 10 ⁵

Table (3):- The occurrence of different bacteria isolates from Sudanese banknotes:

Types of Bacteria	Value of Banknote (SDG)					Total	Contamination percent %
	2	5	10	20	50		
<i>Staphylococcus aureus</i>	4	6	3	4	4	21	42 %
	40%	60%	30%	40%	40%		
<i>E.coli</i>	5	-	4	1	2	12	24 %
	50%	-	40%	10%	20%		
<i>Klebsiella pneumonia</i>	1	3	-	4	1	9	18 %
	10%	30%	-	40%	10%		
<i>Proteus mirabilis</i>	1	-	-	2	1	4	8 %
	10%	-	-	20%	10%		

<i>Pseudomonas aeruginosa</i>	3	3	-	2	2	10	20 %
	30%	30%	-	20%	20%		
<i>Salmonella</i> spp	4	1	3	1	-	9	18 %
	40%	10%	30%	10%	-		
<i>Shigella</i> spp	2	2	4	2	-	10	20 %
	20%	20%	40%	20	-		
<i>Bacillus</i> spp	3	3	2	-	2	10	20 %
	30%	30%	20%	-	20%		
<i>Streptococcus pyogenes</i>	-	4	2	-	1	7	14 %
	-	40%	20%	-	10%		
<i>Niesseria gonorrhoeae</i>	1	2	3	-	1	7	14 %
	10%	20%	30%	-	10%		
<i>Acinetobacter</i>	4	1	-	1	4	10	20 %
	40%	10%	-	10%	40%		
<i>Staphylococcus epidermidis</i>	5	3	2	4	2	16	32 %
	50%	30%	20%	40%	20%		
Total	33	28	23	21	20	125	
	66%	56%	46%	42%	40%		

Results of Questionnaires data:-

Table (4):- Questionnaires data:

Banknotes handlers	Answer	Number of Handlers	Percent %
Education	Yes	47	94%
	No	03	06%
Wash their hands after using a toilet and before dealing banknotes again	Yes	32	64%
	No	18	36%
Wash their hands after using banknotes and before food consumption	Yes	36	72%
	No	14	28%
Handlers knowledge about contaminated banknotes as being a source of some diseases	Yes	38	76%
	No	12	24%
Use of protective measures for a account process in the work place	Yes	09	18%
	No	41	82%

Discussion:-

Fifty samples of Sudanese banknotes (10 samples from every Sudanese banknotes denominations) were analyzed for bacterial contamination, these were 12 different bacterial types identified from banknotes.

All samples were contaminated (100%), this result agrees with reports from other countries which elucidated that banknote are usually contaminated by microorganisms (Basavarajappaet, *et al.*, 2005).

The mean of total viable count of bacteria for samples of banknotes were between ((4.5 – 8.3) x 10⁵ CFU/cm²), and percentages of contamination for banknotes samples were identified to be *Staphylococcus aureus* 42%; *E.coli* 24%; *Klebsiella pneumonia* 18%; *Proteus mirabilis* 8%; *Pseudomonas aeruginosa* 20%; *Salmonella* spp 18%; *Shigella* spp 20%; *Bacillus* spp 20%; *Streptococcus pyogenese* 14%; *Niesseria gonnorrhoeae* 14%; *Acinetobacter* 20%; and *Staphylococcus epidermidis* 32%; as showed in (table 3). These results explain the concept of banknotes act as a potential source of different diseases.

Banknotes were contaminated with more than one bacteria, on the other hand the results showed that high denomination of banknotes such as 50 SDG were less contaminated with bacteria with percentage 40%, compared to low values 20 SDG, 10 SDG, 5 SDG, and 2 SDG, with percentage of 42%, 46%, 56% and 66% respectively.

It was also mentioned by (Uneke, and Ogbu, 2007) that banknote which were handled by large numbers of people under a variety of personal and environmental conditions were usually highly expected to be contaminated, and so the low banknotes denomination which were wide spread and exchangeable between people in the community.

These results agree with comprehensive survey which found that 83% of Europeans agreed that cash contains a lot of bacteria, and that 57% of respondents believed banknotes and coins to be the least hygienic item over other communal materials polled (Basavarajappa, *et al.*, 2005).

More handling and frequent exchange lead to more contamination banknotes, and can be a source of infection associated with oral, nasal, skin and fecal contamination as stated by (Guerin, *et al.*, 2003).

From statistically analyzed questionnaires there were 94% of handlers at different levels education. 64% of the handlers wash hands after using toilets and before dealing with banknotes again. 72% of the handlers wash hands after dealing with banknotes and before food consumption. 76% of handlers have knowledge about contaminated banknotes as being a source of some diseases. 18% of handlers use protective measures for account process in their work place.

To minimize contamination level in banknotes, we needed health promotion for community about the personal hygiene and proper methods to handling of banknotes to avoid diseases that might be transmitted through the circulation process (Igumber, *et al.*, (2007).

Conclusion:-

The study showed pathogenic types of bacteria identified from samples of Sudanese banknotes, according to the study, the low denominations of banknotes were more susceptible to contamination.

Contamination rates of Sudanese banknotes can be attributed to the handlers work type and to the way of handlers were kept the banknotes, some people have been found kept their banknotes in absurd places like in their socks and shoes *et cetera*.

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