ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study of the effect of selected commercial detergents (Soap, Wet wipes) and probiotic Lactobacillus against bacteria isolated from paper currencies in Baghdad, Iraq.

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Manuscript Info

Manuscript History:

Received: 11 January 2015
Final Accepted: 15 February 2015
Published Online: March 2015

Key words:

Soap, Wet wipes, Lactobacillus , Vitek-2 , Baghdad, Currencies.

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Abstract

Background: every day we handle paper currency , its widespread use and constant exchange make currency a likely agent for disease transmission. Therefore, there is unmet need to find new antimicrobial agents from natural sources such as Cell-Free Supernatant from Probiotic Lactobacillus instead of commercial detergents Which had demonstrated inadequate efficiency in this study.

Aim: This study aims to compare between the antibacterial activity of commercial detergents (soap & wet wipes) with Cell-free supernatants from probiotic Lactobacillus against the bacteria that were isolated from currency. At the same time gives an idea of the extent of bacterial contamination of the currency in Baghdad-Iraq.

Methods: A total of 32 samples of Iraqi paper currency, consisting of four denominations (1000,5000,10.000,25.000) ID, gathered aseptically from different sources (Collected from the people most in handled with currencies) street food vendors, cafeterias , roadside vendors and Taxi drivers in Baghdad city.

The isolates were diagnosed by Vitek-2 system, using agar well diffusion method, three materials (soap, wet wipes, CFS from probiotic Lactobacillus) were tested against each type of isolated microorganisms.

Results:

In this present study, Maximum inhibitory activity was observed in the CFS from probiotic Lactobacillus against all isolates, but some isolates showed resistance to commercial detergents (soap and wet wipes).

Conclusions: The study suggests that the Cell-free supernatants from probiotic Lactobacillus possibly could be used as cost-effective source of bioactive compounds that has could be used as bio-preventative agent against Bacteria on the circulating Currencies alternative to commercial detergents.

INTRODUCTION

The environment plays important role in transmission of microbial agents to humans with many environmental materials serving as vehicles **(** Rafiqkhan *et al*.,2013). paper currency is widely exchanged for goods and services in countries worldwide (patrick *et al*.,2010; suaad *et al*.,2011).

An individual living in unhygienic conditions having unhygienic habits will contaminate the currency with bacteria e.g. habits such as using saliva to count the paper currency also leads to contamination and these currency will act as a vehicle delivering bacteria to contaminate the hands of the next user the currencies acts as a tool for easy transfer of bacterial and thus cross contamination takes places (sushil *etal*.,2011).

Generally ,people are not used to wash their hands after handling currency and all most people are not conscious about the fact that they may be affected by many dangerous disease caused by pathogenic bacteria , transmitted to them by handling of currency *(*Hosen *etal*.,2006).

Lactic acid bacteria (LAB) are a group of gram-positive bacteria , non-spore forming , cocci or rods which produce a major end product during the fermentation of carbohydrates*(* Rattana *etal*.,2010).

The majority of microorganisms used as probiotics (consumption of food containing live bacteria is the oldest and still most widely used way to increase the number of advantageous bacteria called probiotics) *(* Giang*etal*.,2011). Belongs to the LAB, within the group of LAB , *lactobacilli* species are the most commonly utilized group of microorganisms due to their potential beneficiary properties as probiotics and have antagonistic activities against large number of pathogens *(* Akhter *etal*.,2013).

Although several studies on the hygiene status of some the worlds currencies have been carried out elsewhere , data on the microbial contamination of currency in Iraq is scanty.

Therefore, the study therefore was focus on the level of bacterial contamination of the currency in Iraq and particularly in Baghdad city , so as to determine whether or not effect the cell free supernatant (CFS) of *Lactobacillus* and some detergents on these bacterial isolates.

Materials and Methods

Sample collection :

A total of 32 Iraqi currencies (paper currencies only) from different places of Baghdad . from people of various categories and to constant exchange with currencies i.e., Taxi drivers , roadside vendors , street food vendors, cafeterias , and students (of collage science , Al-mustansiriyha university), (Bank for control) were collected .

Paper currencies of (25.000 , 10.000 , 5000 , 1000) Dinars Iraqi(DI). Were collected for microbial analysis.

Inoculation of samples:

Swaps from paper currencies were directly inoculated in nutrient broth and allowed to incubate for 24 hrs at 37 C^o . after 24 hrs 0.0l loopfull of the broth from each samples were inoculated on nutrient agar plates and incubated at 36C^o for 24 hrs. the bacterial colony was prepared to identify.

Identification of the bacterial isolates:

The bacterial isolates were allowed to grow on the selective media MacConkey agar , Mannitol salt agar . from each sample , serial dilution was performed to get a single colony for identification by Vitek-2 system according to the manufacturer's instructions. As end or final identification.

1) Lactobacillus isolate : *Lactobacillus acidophilus* , from high studies laboratories / Biology Department / Science collage / Al-mustansiriyha University , lactobacillus isolate from cucumber identified was taken.

2)preparation of Cell- Free supernatants :

Isolates to be tested for antimicrobial activity were inoculated in MRS broth for 48 hrs at 37C^o .

Bacterial cells were removed by centrifuging the culture at 5000 g for 20 min at 4C^o , The PH value of supernatants were adjusted to PH 6.5-7.0 by the addition of 1N NaOH , the supernatants were membrane filtered *(*Reza *etal*.,2012).

-Soap of test :

To perform this experimental study , soap of common use from shop was purchased and its dilution was made (1 gm : 100 ml) for testing the bacterial activity of different organisms. The soap used was **WEST** (Medicated soap) made in Dubai UAE.

-Wet wipes of test :

Also **Cleanic** wet wipes (Antibacterial wipes) made in EU was squeezed in sterilized plate and its produced solution was made for testing the bacterial activity of different organisms.

-Assay of Antimicrobial Activity:

The inhibitory activity of supernatant of *lactobacillus* and detergents (soap and wet wipes) was screened against isolated organisms using conventional Agar – well diffusion .

Briefly , indicator bacteria were grown overnight and spread onto the Muller-Hinton Agar plate , then 5mm diameter wells were punched into the surface using a sterile borer.

Subsequently CFS of lactobacillus and (soap solution and wet wipes squeezer) were added to each well of the plate and incubated at 37C^{o} for 24hrs .

The antimicrobial activity was recorded as growth free inhibition zones (diameter) around the well. Distill water served as control.

Results and Discussion

Microbial examination was carried out for (32) paper currency in which all the currency paper were not contaminated with microorganisms (Table 1).

In Iraq , poor –currency – handling culture is widespread , and there is an indiscriminate abuse of paper currency, a great majority of the populace does not carry money in wallets and squeezing of paper currency is a common occurrence .

These activities not only enhance currency contamination but may also increase the risk of infection from contaminated currencies. *(* Pradeep *etal*.,2012)

Among many studies , (Md. Shakir Uddin Ahmed *et al* ., 2010) suggested that the paper currency commonly contaminated with pathogenic microorganisms and this contamination may play a significant role in the transmission of potentially harmful microorganisms or different disease such as cholera , diarrhea ,skin infections and also poses antibiotic resistant.

Table (1) : Data acquired on currency papers :number and types of microorganism in each type of paper currency .

Paper currency / Microorganisms	25.000	10.000	5000	1000
Sphingomonas paucimobilis	0	0	2	4

Leclercia adecarboxylata	0	2	2	6
pantoea agglomerans	2	2	2	0
Enterobacter cloacae	2	2	4	2

The pathogenic bacteria were confirmed as *Sphingomonas paucimobilis, Enterobacter cloacae,Leclercia adecarboxylata ,pantoea agglomerans.*

Most of the dirty paper currencies which were collected mainly from roadside vendors were found to be carring most the pathogenic microorganisms , this is may be due to the position of the moisture on the paper currencies which provides a favorable condition for the microorganisms to grow on it and carbon source *(* Debajit *etal*.,2012).
Also in Table (1) : we found paper currency with Iraqi Dinar (1.000) yielded the largest number (12) pathogen , paper currency with Iraqi Dinar (5000) was the middle in yielding with (10)pathogen.
In other side , Iraqi currency with (10.000) yielded (6) all three microorganisms except one (*Sphingomonas paucimobilis*) .
while currency with Iraqi Dinar (25.000) was the only currency which yielded (4) microorganisms just.
Our experiments showed that the Iraqi paper currency (1.000) ID allows growth and transmission of pathogens.
This is theory could contribute to the transmission of microorganisms within the Iraqi community, this currency consider more circulation among people, especially in roadside vendors , this phenomenon may play a role in the transmission of pathogens within the community because of money is frequently touched during daily life *(* Habip *etal*.,2013).
It has been shown that currency provides a surface area for microbe establishment and function as a strong vehicle in transmitting microbes as well , so that depending on where the money has been passed through its microbial flora will change *(* Mehdi *etal*.,2011).
Bacterial agents found to contaminate our local currency notes included Enterobacter cloacae can cause lower respiratory tract infections , urinary tract infections ; However , recent report show that it can be an invasive pathogen (Suaad *et al*.,2011; Sushil*et al*.,2011; Abdulmoneim*et al*.,2010).
Smaller unit currencies appeared to be more highly contaminated than larger unit currencies, probably because the small unit currencies are most frequently handled in petty , daily monetary transactions *(* Neel *etal*.,2012).
Many studies agreed with our result about the currencies contamination in India*(Rafiqkhan etal*.,2013), Bangladesh*(Md.shakir etal*.,2010), Ghana*(Patrick etal*.,2010) , Saudi Arabia*(Al-Ghamid etal*.,2011) , Iran*(Mir-M Hassan etal*.,2013) ,Palestine*(Hazemetal*.,2012) , Nigeria*(Adamu etal*.,2012) , Mecca*(Samy etal*.,2012),Iraq*(Huda etal*.,2012) .

The results from testing the effect of three types : natural CFS and commercial disinfectants selected (West soap and Cleanic wet wipes) on the growth of bacteria isolated from currencies showed that the liquid soap was able to disinfect paper contaminated with *Leclercia adecarboxylata* but not other isolates .
The wet wipes showed no effect the isolated *pantoea agglomerans.*(Table 2) but not *Sphingomonas paucimobilis,leclercia adecarboxylata, Enterobacter cloacae* .

Finally ,the results of this study confirmed that CFS of Lactobacillus showed a good inhibition activity; we found that 100% of our tested isolated organisms submitted to inhibition by CFS .
This a higher than that previously materials (soap and wet wipes).
Figure 1.2.3.4.

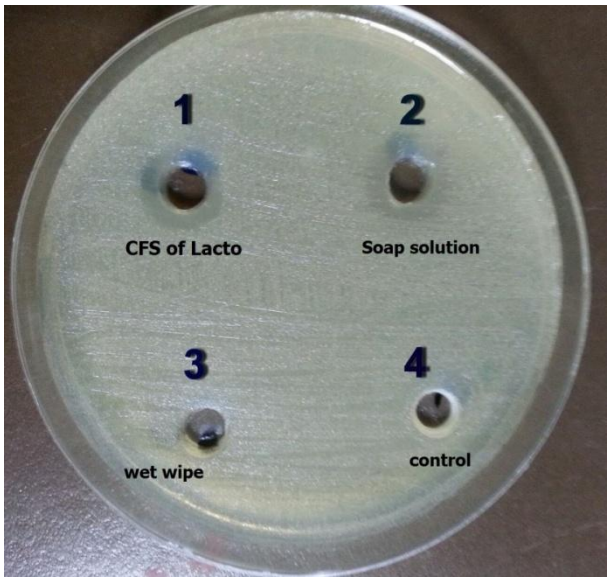

Figure 1: Antibacterial activity of CFS of lactobacillus , Soap solution and wipes squeezer against *Enterobacter sp*.

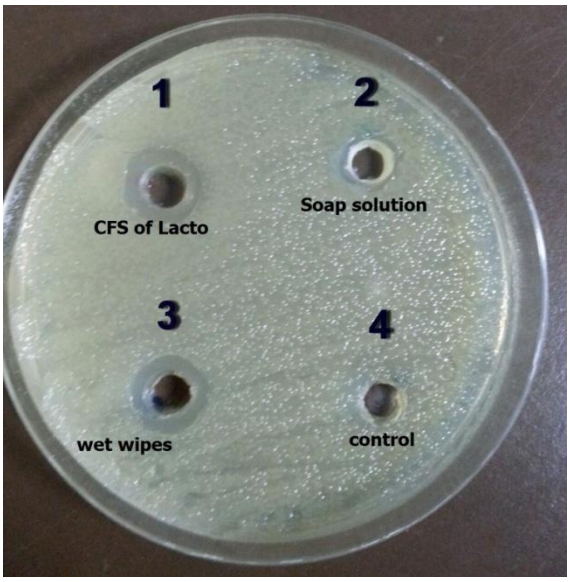

Figure 2 : Antibacterial activity of CFS of lactobacillus Soap solution and wipes squeezer against *Leclercia sp.*

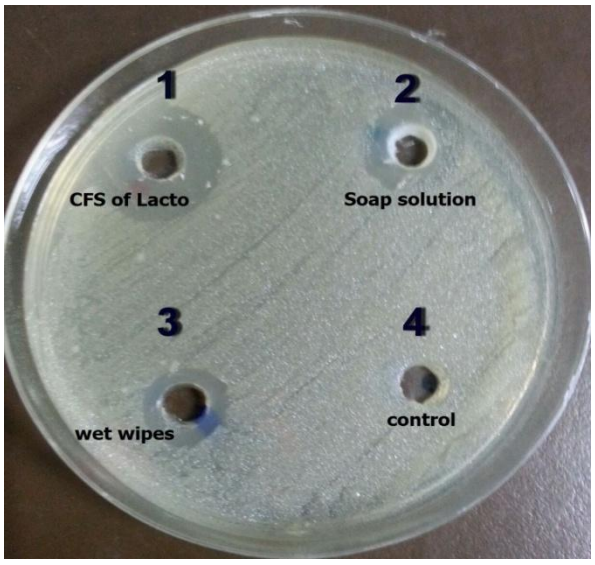

Figure 3: Antibacterial activity of CFS of lactobacillus , Soap solution and wipes squeezer against *Sphingomonas sp*.

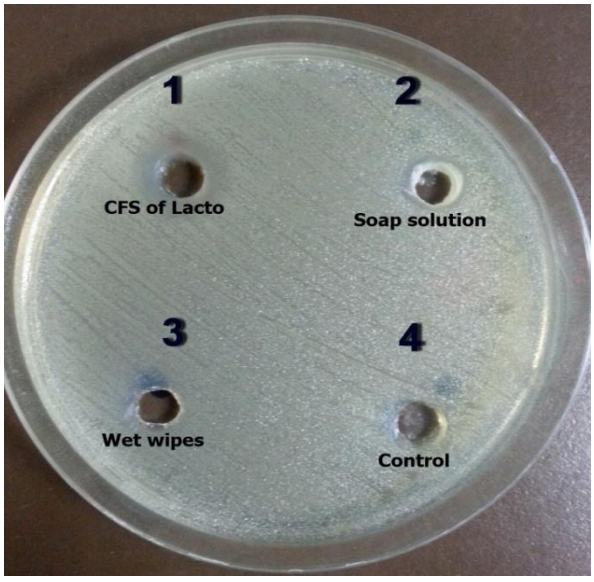

Figure 4: Antibacterial activity of CFS of lactobacillus, Soap solution and wipes squeezer against *pantoea sp.*

Table (2) : The antibacterial activity of detergents (soap & wet wipes) and CFS Lactobacillus against currency paper isolates.

Isolates	Antibacterial activity		
	CFS	Wet wipes	soap
	Zone of inhibition(mm)		
Sphingomonas paucim 1	14	11	–
2	22	18	12
3	14	10	14
4	16	12	16
5	16	18	12
6	22	11	–
Pantoea agglomerans 1	15	–	12
2	16	–	–
3	17	–	–
4	17	–	11
5	14	–	10
6	15	–	12

Leclercia adecarboxylata 1	**17**	**11**	**12**
2	**16**	**11**	**11**
3	**15**	**10**	**10**
4	**15**	**16**	**12**
5	**16**	**10**	**12**
6	**17**	**12**	**14**
7	**18**	**8**	**9**
8	**14**	**12**	**13**
9	**15**	**14**	**9**
10	**15**	**10**	**16**
Enterobactercloacae 1	**15**	**11**	**10**
2	**16**	**15**	**9**
3	**13**	**12**	**—**
4	**11**	**15**	**—**
5	**17**	**11**	**13**
6	**17**	**9**	**—**

7	**14**	**17**	**—**
8	**15**	**12**	**15**
9	**13**	**17**	**7**
10	**17**	**9**	**10**

***1,2,3,4,5,6,….. : Isolates Number**

-soaps are generally used for the removal of germs and for cleaning purpose.

Soaps usage is very common and now a days especially antibacterial soaps are very popular *(*Saba *etal*.,2009) ., so with the use of antibacterial soaps are bacteria killers. Because soap contain material that can kill the germs *(Amani etal*.,2010).,but not killing all microorganisms *(Aliyu etal*.,2012).

In the other side , the wet wipes also very important of hand hygiene .Alcohol- based hand sanitizers have been recommended by the CDC for routine de- contamination of hands when soap and water are un – available *(Natalie etal*.,2010).

The wet wipes superior efficacy in removing microorganism is attributed to the higher ethanol concentration *(David etal*.,2013).

Application of wipes could therefore, be very useful and accepted to play significant role in the health care facilities and households , as it reduces the bioburden on the hand and as well as prevents irritation due to constant hand washing (Ahmed *et al*.,2014; Janice *et al*.,2008).

In despite of the higher efficacy of wet wipes but they're not were on all microorganisms like soap.

In our present study CFS was the alone killer to all microorganisms and in high inhibition concentration . and did not fail in any one, this activity due to presence many compounds include many organic acids such as lactic, acetic and prop ionic acids which provide an acidic environment unfavorable for the growth of pathogenic microorganisms *(Ashokkumar etal*.,2011).

Also these acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transpore, reducing intracellular PH and inhibiting a variety of metabolic functions (Abdelkader *et al*.,2013).

The reason for we chose the CFS of Lactobacillus as antibacterial is own is Avery broad mode of action and inhibit both gram-positive and gram-negative as well as yeast and molds (Rattanachaikunsopon *et al*.,2010; Jagoda*et al*.,2010).

In addition to acids, these strains can produce a range of other antibacterial metabolites such as etchant from the heterofermentative pathway , H2O2 produced during aerobic growth and diacetyl which is generated from excess pyruvate coming from citrate (Abdallah *et al*.,2013; Wadhai *et al*.,2011).

Conclusion

-In this work we focused on contamination of currency papers obtained from various group of people belonging to weaker social and economic status living in Baghdad city, Iraq.

- Our results showed that some detergents like (soap & wet wipes) not efficiency for remove germs that isolated from currency.

-CFS of lactobacillus have a higher antibacterial activity with range 100% against microorganism.

Recommendations

Therefore, a recommendations should be pointed out such as a awareness of people is how to handle money to reduce their hand contamination so reducing currency pollution

There should be public awareness of the fact that currency papers could be a source of infection and could be dangerous to health .

We recommended that should make a natural wet wipes from *Lactobacillus* compounds to get a guaranty 100% from not contamination.

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