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Full Length Research Paper

Influence of moisture content on some physical properties of pineapple pomace based mash

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The physical properties of pineapple pomace are important in designing and fabricating equipment and structures for handling, transporting, processing and storage. The study was conducted to investigate some physical properties of pineapple pomace at various moisture levels. The experiment was used to evaluate the physical properties at different pineapple pomace/cassava flour ratio mash (5:1, 6:1 and 7:1) and the moisture content of the mash (12.36, 15.66, 19.58, 22.70 and 26.26%). In the same moisture range, the bulk density increased from 427 to 679 kg/m^3. The coefficient of friction of pineapple pomace flour and cassava at various mixing ratio increased linearly (p<0.05) for all the surfaces and varied with structural surface in the moisture range of 12.36 to 26.26% (dry basis). The maximum and minimum values of coefficient of friction were obtained on the surface of wood and stainless steel respectively. Pineapple pomace moisture content effect was statistically significant (p<0.05) on all properties investigated while baseline data were generated for the development of necessary handling and processing equipment. The results encourage value addition to the industrial waste while reducing environmental pollution.

Key words: Designing, processing, mash, surface, steel.

INTRODUCTION

Pineapple is the world's most popular non-citrus tropical and subtropical fruit known for its excellent sensory attributes and nutritional composition (Azevedo et al., 2007). It shows great potential for the processing industry due to its high contents of vitamins C and A. The residues from its processing called pineapple pomace constitute heterogeneous mixture of husks and skins, accounting for 4 to 12% and 15 to 25%, respectively of the total mass of pineapple produced (Mantovani et al., 2004; Rogério et al., 2007). Traditionally, cassava is mostly used as human food but it is also an important raw material in its various forms for many textile, paper,

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and pharmaceutical industries; battery manufacturers need starch and its derivatives. The substitution of wheat or maize flour partially with cassava flour and production of cassava chips and pellets for animal feed are other areas of utilization with some potential significant in Nigeria.

The present methods of handling and processing the pineapple pomace are very slow and full of drudgery with a lot of wastage. Therefore, it is necessary to develop better means of processing the pineapple pomace into useful products using suitable machines and equipment. Knowledge of its physical properties is very useful to machine design. The increased awareness of the nutritive and economic benefits pineapple pomace provides in livestock production must be taken into consideration to avoid potential market saturation. Most of the investigations show that the physical properties of agricultural products are moisture dependent. Although most research work in the field of the coefficient of friction of agricultural materials is related to grains, some literature was found regarding the static coefficient of friction, adhesion coefficient, and cohesion coefficient of forage materials. Coefficients of friction of grains and forage materials are significantly affected by material moisture content. Several researchers (Sadiku and Bamgboye, 2014; Aviara et al., 2012; Oduntan et al., 2010; Dash et al., 2008; Naderiboldaji et al., 2008; Afzalinia and Roberg, 2007; Owolarafe et al., 2005) studied the physical properties of different agricultural products for the above purpose. Therefore, in order to increase supply, it is necessary to modernize production techniques and optimize processing conditions (Audu et al., 2004). To achieve this, adequate information on the properties of pineapple pomace is required for the design of equipment for its. No work however, appears to have been carried out on the moisture dependence of physical properties of this important fruit pomace. Data of physical properties of pineapple pomace were required as input to the design calculations of the process equipment of this materials. Therefore, this study investigated the influence of moisture content on the properties of pineapple pomace for future design of processing equipments.

MATERIALS AND METHODS

Sample preparation

The fresh pineapple pomace sample, rich in water (about 90%) was obtained from a juice processing plant (Funman Agricultural Products Ind. Ltd, Moor Plantation Ibadan). Two groups of 250g sub-samples of the pineapple pomace were used for the determination of the proximate. Analyses of the samples were carried out in triplicate according to AOAC standardized procedures and the average values of the observation were documented.The pineapple pomace was dried in a fluidized bed dryer (Fexod AS 230, Nigeria) at temperature of 65°C to avoid changes in the functional properties and proteins with air velocity of 5.0 m/s in a forced convection thin layer dryer with thickness of between 10 and 15 mm. The dried samples were ground to powder through a screen plate in the disc mill (Model 206, Fexod disc mill, Nigeria).

Experimental procedure

Samples of the pineapple pomace and cassava flour as a binder were ground separately with a 3 mm screen disc mill (Model 206, Fexod disc mill, Nigeria). Particle size distribution of the ground samples was determined separately according to ASABE Standard S269.3 (ASABE, 2003). Hundred gram of the material was placed on the top sieve of nest of successively smaller sieves. The set consists of six test sieves and a pan with aperture sizes ranging from 0.1 to 2 mm (Endecotts Limited, London). The nest of test sieves was clamped on a shaker for 10 min after which the mass of material retained on each sieve was measured on a digital scale (Model Ultra – 75, U.S.A). Cumulative weight of each fraction was obtained as percentage of total mass of the sample.

Bulk densities of the wet pineapple pomace, dried pineapple pomace and cassava flour were determined as recommended by ASABE S269.4 (ASABE, 2003). A container was filled with pomace using a funnel, without compacting the content. The material was leveled with the top surface of the container and weighed. The mash bulk densities were obtained from the ratios of the measured masses of samples in the container to the volume of the container. Five measurements of each experimental run were taken to obtain the average values and standard deviations.

The moisture content of the pineapple pomace and cassava flour was determined separately using the oven method as described in Equation 1 (ASABE, 2003). Moisture cans were weighed on an electronic weighing machine (Model Ultral-75, U.S.A) and recorded.

$$MC = [(W_i - W_f)/ W_i]100\% \quad (1)$$

Where, MC = moisture content; W_i = initial weight; W_f = final weight. The coefficient of sliding friction of the samples was determined on four various surfaces (polished wood, mild steel, galvanized steel and stainless steel) at different pineapple pomace and cassava flour ratio. The incline plane was gently raised and the angle of inclination at which the sample start to slide was read off the protractor with sensitivity of one degree. The tangent of the angle was recorded as the coefficient of friction.

The static angle of repose of the samples was determined using a cylindrical container open at both ends and placed on a flat surface (Aviara et al., 1999). It was filled from the top with samples. The cylinder was then lifted up gradually allowing the sample to flow and form pile. The angle of repose was calculated from the measurements of the vertical depth and radius of spread of the sample. This was replicated five times.

RESULTS AND DISCUSSION

Particle size distribution

The particle size distribution of the pineapple pomace sample after grinding is shown in Table 1. It is apparent that a large part of the pomace flour was retained on the 0.6 and 0.8 mm nominal sieve size which was about 50% of the total sample. Some samples are quite fine on the 0.1 mm sieve size. Similar results had been observed in the particle size determination of peanut pomace (Fasina,

Table 1. Particle size distribution of pineapple pomace.

Nominal sieve size (mm)	Sample retained (g)	Sample retained (%)	Cumulative distribution (%)
2.0	5.75	5.75	100.00
0.8	34.14	34.14	94.25
0.6	12.83	12.83	60.11
0.5	9.14	9.14	47.28
0.3	25.60	25.60	38.14
0.1	12.54	12.54	12.54
0.0	0.00	0.00	0.00

Table 2. Mean values of the bulk density of pineapple pomace mash (kg/m^3) at different moisture content.

Pineapple pomace/ cassava ratio	Pineapple pomace flour mash moisture content, % d.b				
	12.36	15.66	19.58	22.70	26.26
1:0	427±19	468±15	485±6	516±9	547±11
5:1	560±24	597±22	629±17	664±29	695±18
6:1	535±26	583± 33	632±12	658±29	681±22
7:1	496±19	542±12	584±19	617± 27	679±18

2008). The result shows that pineapple pomace particles have larger diameter which might affect the texture and uniformity of the product. According to Frame (1994), larger particles of material have less contact area with the barrel of the extruder during the period when are convey along the screw and are less affected by the barrel temperature than fine particles.

Bulk density

The variation of pineapple pomace/cassava flour mash ratio bulk density with moisture content is shown in Table 2. The bulk density of pineapple pomace/cassava flour mash ratio at 1:0, 5:1, 6:1 and 7:1 increased from 427 to 547kg/m^3, 560 to 695 kg/m^3, 535 to 681 kg/m^3 and 496 to 679 kg/m^3 as their moisture contents increased from 12.36 to 26.26% (db) respectively which indicated low bulk density. From this table, it can be seen that the bulk density of the pineapple/cassava mash ratio is lower at higher pineapple pomace inclusion rate of 7:1 than that of higher cassava flour inclusion rate of 5:1 within a similar moisture range.

The relationship existing between pineapple pomace/ cassava flour bulk density and moisture content can be expressed with the following equations:

$\rho_{1:0}$= 8.24M+ 329.47; R^2 = 0.9829

$\rho_{5:1}$= 9.66M + 442.43; R^2 = 0.9976

$\rho_{6:1}$= 10.55M+ 414.06; R^2 = 0.9743

$\rho_{7:1}$= 13M+ 331.45; R^2 = 0.9910

Where: $\rho_{1:0}$, $\rho_{5:1}$, $\rho_{6:1}$, $\rho_{7:1}$ - bulk densities (kg/m^3) of pineapple pomace/cassava flour mash ratio 1:0, 5:1, 6:1 and 7:1 respectively.M = moisture content (%)

A similar observation was made by Kumar et al. (2010) as they studied the bulk density of fruit pomace. Frame (1994) reported that a high volumetric capacity screw is required for the design of extruder with low bulk density material for their conveying volume rather than pumping ability. It was reported that actual length of feed zone on the screw shaft depends on the bulk density of the pineapple pomace (Frame, 1994).

Coefficient of sliding friction

The mean results obtained for frictional properties of pineapple pomace flour and cassava flour at different inclusion ratio on four structural surfaces at different moisture levels (12.36 to 26.26%) are given in Figures 1 to 5. The effect of moisture content on the coefficient of friction for the inclusion ratio of pineapple-cassava on mild steel, stainless steel, galvanized steel and wood surfaces are significant. Coefficient of friction followed an increasing trend with increasing moisture content as shown in all the figures.

The result indicated that coefficient of friction was

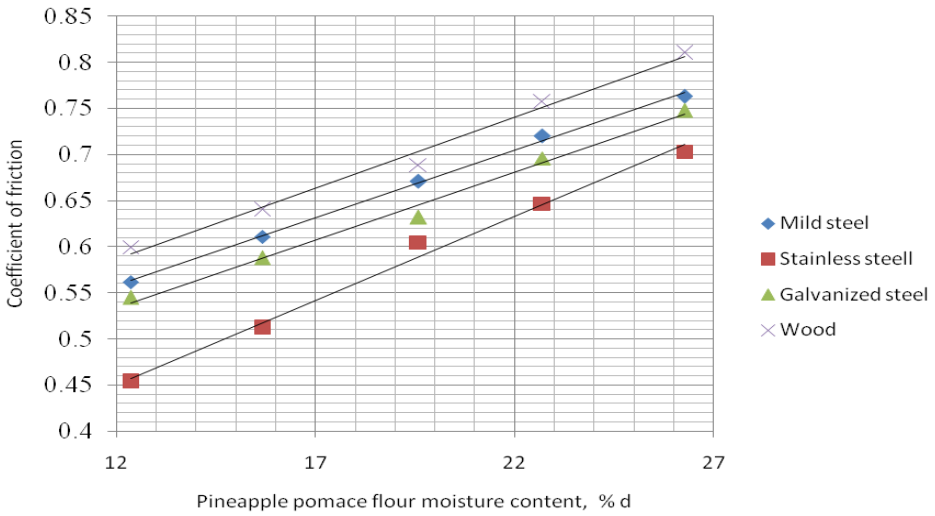

Figure 1. Coefficient of friction variation with pineapple pomace moisture content.

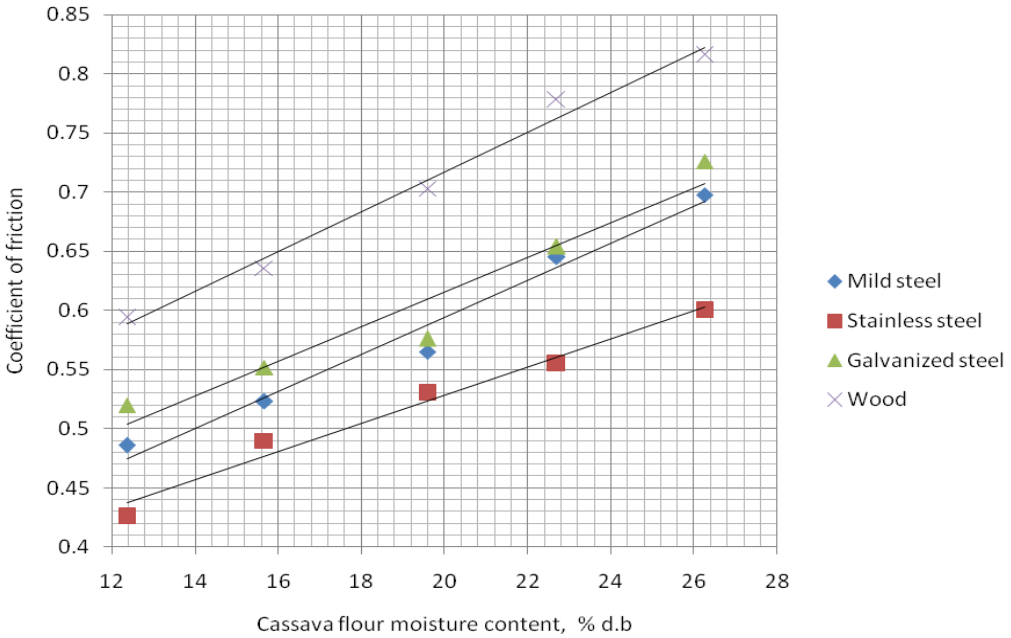

Figure 2. Coefficient of friction variation with cassava flour moisture content.

greater on the wood surface and galvanized steel than mild and stainless steel. The value obtained for pineapple pomace sample was higher than that of cassava flour (Figures 1 and 2). This showed that pineapple pomace flour was not smooth as cassava flour. Figures 3 to 5 showed that coefficient of friction increased with pineapple pomace inclusion rate. The coefficient of friction of pineapple pomace flour and cassava at various mixing ratio increased linearly ($P<0.05$) for all the surfaces and varied with structural surface in the moisture range of 12.36 to 26.26% (db). The maximum value of 0.81and 0.82 were obtained on the surface of wood for pineapple pomace and cassava flour res-pectively. Minimum value of 0.45 and 0.43 were obtained on the surface of stainless iron sheet for pineapple pomace and cassava flour respectively. This result was in good agreement with the results reported for alfalfa and similar materials in the literature (Shinners et al., 1991; Ling et al., 1997; Mani

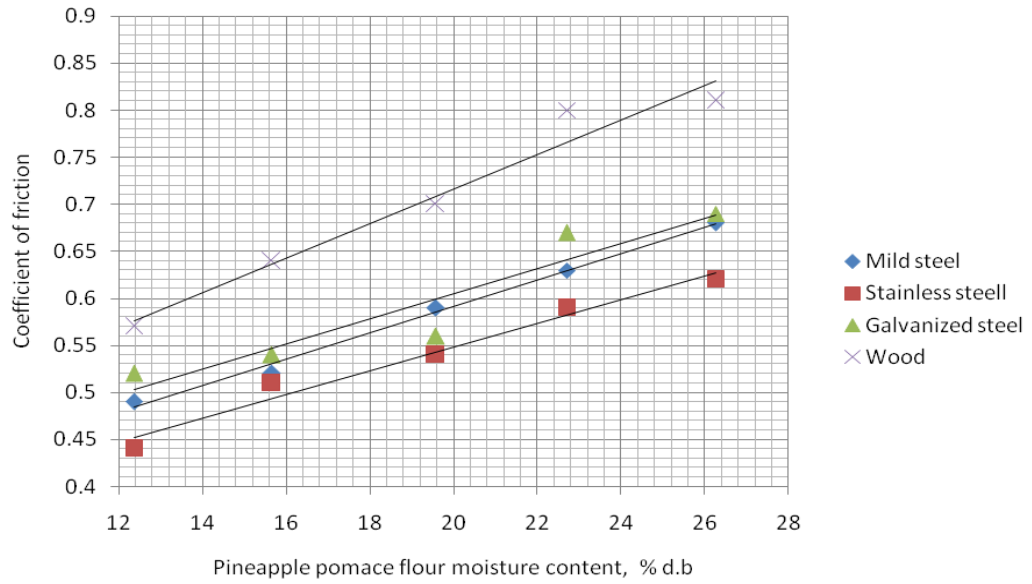

Figure 3. Coefficient of friction variation with pineapple pomace/cassava ratio of 5:1 at different moisture content.

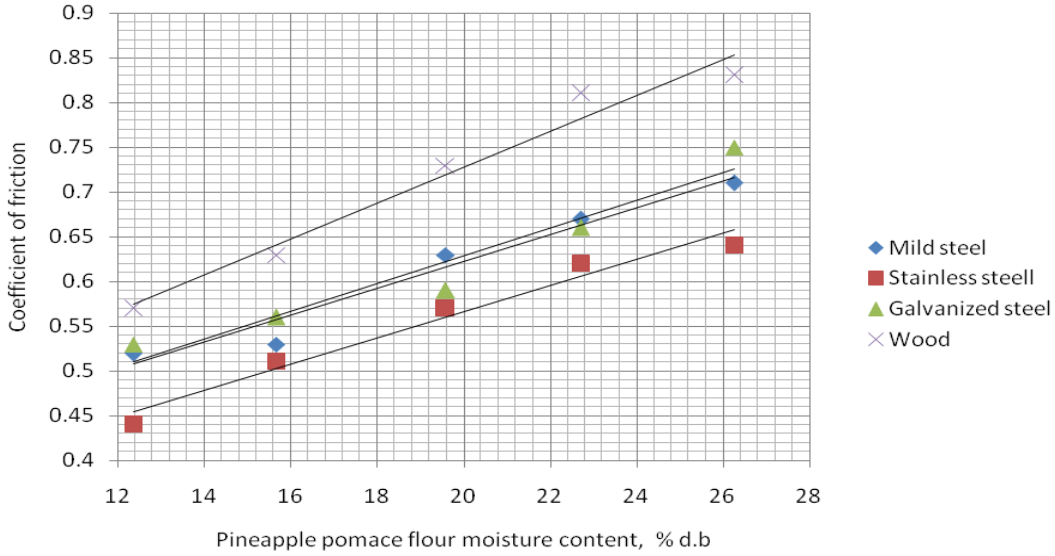

Figure 4. Coefficient of friction variation with pineapple pomace/cassava ratio of 6:1 at different moisture content.

et al.,2003; Afzalinia and Roberge, 2007). Zhang et al. (1994) found that the coefficient of friction of wheat on a corrugated steel surface increased with an increase in moisture content. Ling et al. (1997) stated that the static and sliding coefficients of friction of wood ash increased with an increase in ash moisture content. Chandrasekar and Viswanathan (1999) showed that the coefficient of friction of arabica and robusta (coffee) parchments on various surfaces increased with an increase in moisture content in the range of 9.90 to 30.6% (wb). Aviara et al. (1999) reported a range of 0.41 to 0.98 for the coefficient of static friction of guna seeds in the moisture range of 4.70 to 39.3% (db). The relationship between the coefficient of friction and moisture content can be expressed for different structural surfaces were linear using the following equations:

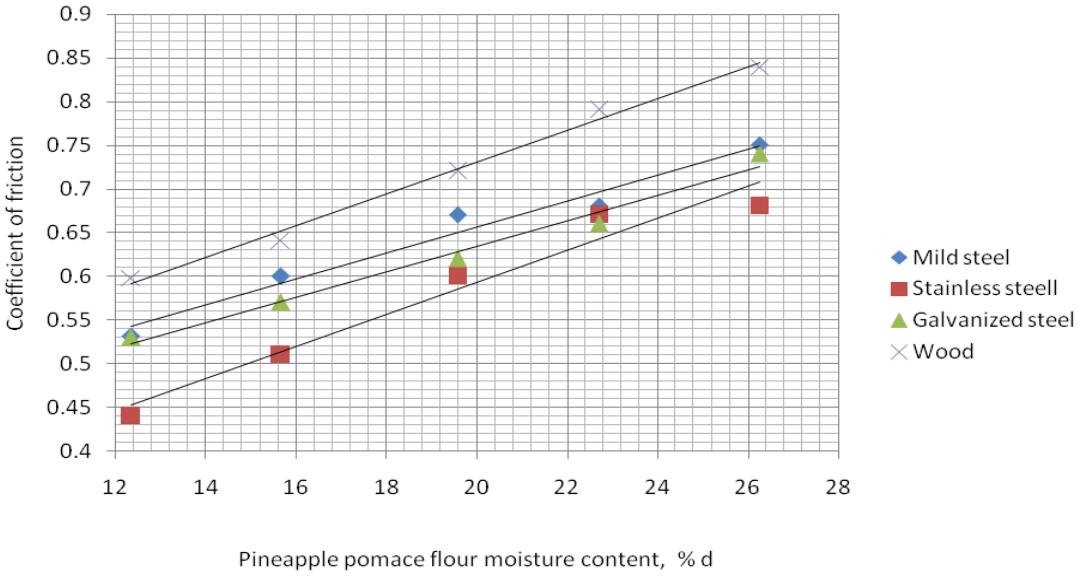

Figure 5. Coefficient of friction variation with pineapple pomace/cassava ratio of 7:1 at different moisture content.

f_{mp}= 0.014M + 0.382; R^2 = 0.948
f_{sp}= 0.018M + 0.232; R^2 = 0.934
f_{gp}= 0.014M + 0.547; R^2 = 0.99
f_{wp}= 0.015M +0.402; R^2 = 0.988
f_{mc}= 0.015M + 0.218; R^2 = 0.975
f_{sc} = 0.011M+ 0.291; R^2 = 0.977
f_{gc} = 0.014M + 0.322; R^2 = 0.937
f_{wc} = 0.016M + 0.380; R^2 = 0.987

Where f_{mp}, f_{sp}, f_{gp}, f_{wp}, f_{mc}, f_{sc}, f_{gc}, f_{wc} - the static coefficients of friction of pineapple pomace and cassava on mild sheet, stainless sheet, galvanized sheet and wood respectively. M - moisture content (%)

Similar findings were reported for baobab fruit pulp powder (Adekunle et al., 2013). The frictional properties are useful in designing partitions, lining materials and in bulk transportation of agricultural products in trucks (Jahromi et al., 2008). However, the coefficient of static friction of agricultural materials depends on the moisture content of produce at the time of testing (Dutta et al., 1988).

Conclusion

The following conclusions were drawn from the results of this research:

(1) The particle size distributions of the dried pineapple pomace sample after grinding have larger diameters.
(2) The bulk density of pineapple pomace/cassava flour mash at inclusion ratio indicated low bulk density. Low bulk density will increase the packing density; reduce the cost of transport and storage space. High efficient machine can be developed with low bulk density.
(3) The highest coefficient of static friction and angle of repose for pineapple pomace flour and cassava at various mixing are on wooden surface and the lowest on stainless sheet surface and therefore, stainless it could be used to minimize abrasion damages and for the construction of processing machine to improve efficiency.
(4) The results encourage value addition to the industrial waste and reduce environmental pollution.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

In vitro characterization of a vancomycin-resistant strain of *Leuconostoc lactis* isolated from chicken carcasses and its activity against some foodborne pathogens

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Recently, increasing attention has been paid on *Leuconostoc lactis* as a promising bioactive organism against food-borne and spoilage bacteria. A total of nine strains, including six different species of the genus Lactobacillus and three species of the genus Leuconostoc, were isolated from chicken carcasses (n=60) collected from wholesale poultry markets located at Al-Riyadh city, Saudi Arabia in 2016 and identified by API 50 CHL assays. *L. lactis* isolates were resistant to bile salts and vancomycin. The autolytic phenotype of *L. lactis* was evaluated under starvation conditions in the presence of potassium phosphate buffer. The strains tested showed partial autolysis of approximately 18% after 7 h of starvation at 37°C at the end of the exponential phase. The inhibitory activity of whole-protein extracts of *L. lactis* against the foodborne bacteria, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus* was evaluated by renaturing sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The major activity of the total protein appeared as two clear bands on the SDS-PAGE, of approximately 57 and 42 kDa against *L. monocytogenes*, *B. cereus* and *B. subtilis.* No active band was shown against *S. aureus* and *M. luteus*.

Key words: Bacteriocins, biopreservation, lactic acid bacteria, pathogens, poultry.

INTRODUCTION

Leuconostoc is a genus of Gram-positive bacteria, placed within the family of Leuconostocaceae. They are generally ovoid cocci often forming chains. *Leuconostoc* spp. are resistant to vancomycin and are catalase-negative, which differentiates them from staphylococci (catalase-positive). *Leuconostoc* bacteria are used as starter culture bacteria, and are similar to other lactic acid bacteria (LAB), because their fermentation of some vegetables and milk products results in a sour odour that is used as an indicator of freshness. Although, some species of the genus are capable of causing infections in humans (Coovadia et al., 1987; Coovadia et al., 1988), *Leuconostoc* may be used to improve food hygiene, safety and shelf-life by producing antimicrobial substances such as bacteriocins, lactic acid and hydrogen peroxidase (Holzapfel et al., 1995; Jay 1996).

Bacteriocins are active against some Gram-positive bacteria and foodborne pathogens, such as *Listeria monocytogenes, Clostridium botulinum* and *Staphylococcus aureus*, and have been proposed to serve as bio-preservatives in foods (Franz et al., 2007). Probiotics are defined as live microorganisms that provide health benefits to the host (FAO/WHO, 2002). Some lactic acid bacteria, including those belonging to the groups *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc,* have been reported to possess active probiotic capabilities (Fuller, 1991; Ogunshe 2008).

Leuconostoc spp. are inhibitory to other bacteria because they produce organic acids and hydrogen peroxide, thereby altering the pH. Bacteriocin is produced during bacterial growth in a suitable medium. As shown by Stiles (1996), although bacteriocin production by *Leuconostoc* spp. was first observed in the 1950s, more extensive studies of bacteriocins produced by *Leuconostoc* spp. were not conducted until 1984, when the antagonistic activity of *Leuconostoc* spp. was reported. Since then, the following bacteriocins have been identified: Mesentericin Y105 produced by *Leuconostoc mesenteroides*; leucocin A-UAL 187 produced by *Leuconostoc gelidum;* carnosine 44A produced by *Leuconostoc carnosum*; and leuconocin S produced by *Leuconostoc paramesenteroides*. Stiles (1996) reported that bacteriocins produced by Leuconostoc may or may not be active against other lactic acid bacteria but exhibit varied effects against *Listeria*. Mesentericin Y105 is active against *Listeria* spp., and the amino acid sequences for leucocin A and mesentericin Y105 have been determined. Only two amino acids in the antibacterial protein sequences of these two bacteriocins were found to be different.

The aim of the present work was to identify and characterize commensal bacteria isolated from chicken carcasses, focusing especially on *Leuconostoc lactis* and its activity against some Gram-positive foodborne bacteria.

MATERIALS AND METHODS

Whole chicken carcasses (n = 60) were obtained from a wholesale poultry market located in Saudi Arabia. Chicken carcasses were approximately of 1.5 kg ± 150 g weight and transported under controlled cooling temperatures (2°C) at the Food Microbiology Laboratory, Department of Food Science and Nutrition, College of Food and Agricultural Sciences, King Saud University, until further use.

Microbiological analysis

The isolation of lactic acid bacteria from chicken carcasses was performed by routine microbiological isolation procedures with serial dilutions on selective media of Man Rogosa Sharpe (MRS) agar (Oxoid CM115, Basingstoke, Hampshire, UK) supplemented with 0.25% (w/v) L-cysteine CHR (Fluka AG, Buchs SG- K595-/88/4./8). Whole chickens were washed in 0.1% lactose broth medium (Oxoid CM0137), shaken manually for 1 min and then serially diluted at 1/10 proportions with 0.1% lactose broth. One millilitre of each sample dilution was plated onto Petri dishes and covered with MRS agar that had been sterilized and cooled to 50°C. The plates were incubated for 72 h at 37°C in an anaerobic system (BBL Gas Pak, Becton Dickinson and Co., Cockeysville, MD 21030, USA). Suspected colonies were selected from MRS agar plates of the 10^{-5} dilution (5 log CFU). Pure colonies were also randomly selected from the primary plates after incubation and preserved in 15% glycerin at -20°C for stock and bench cultures. Colonies were identified using the API 50 CHL kit (bioMerieux, Marcy l'Etoile, France).

Biochemical tests

Biochemical tests were performed using API 50 CH and API CHL test kits (Bio-merieux), according to the manufacturer's instructions. The ability of a strain to ferment 49 carbohydrates after 18 h of growth in MRS broth and change the colour of the bromocresol purple indicator from purple to yellow indicated a positive result. Susceptibility to vancomycin was evaluated by the disc diffusion method on Mueller-Hinton agar (CM 0337) with antibiotic discs containing 30 µg of vancomycin (Oxoid CT00588), ampicillin 10 µg (CT 0003B as a positive control and according to the National Committee for Clinical Laboratory Standards (NCCLS) (2002).

In the present study, in order to examine the possibility of *L. lactis* to be a Gram-positive pathogen, apart from the API 20 STREP and API 50 CHL kits (bioMerieux, Marcy l'Etoile, France) that led to the

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identification of vancomycin-resistant, catalase-negative Gram-positive species, additional phenotypic assays, with assistance of a genotypic confirmation were run, confirming its non-pathogenic character (results not shown). However, molecular identification data on *L. lactis* are not provided in the present study.

Assessment of antimicrobial activity by well diffusion assay

The antimicrobial activity of *L. lactis* (whole crude protein of the cells) against *L. monocytogenes*, *Bacillus cereus* and *Bacillus subtilis*, *S. aureus* and *Micrococcus luteus* was determined through the well diffusion assay. All the tested bacteria were incubated in nutrient broth at 37°C for 24 h. Twenty millilitres of Muller-Hinton agar were poured into Petri dishes and inoculated with 0.1 mL of the broth from a 24 h culture containing the target bacteria. After solidification, the dishes were stored at 4°C for 2 h. Two wells were made in each dish and filled with 50 µL of different concentrations of cell-free *Leuconostoc* filtrates. The Petri dishes were then incubated at 37°C for 24 h. The antimicrobial activity was detected by the presence of a clear zone around the wells. Similarly, the effect of 50 µL of filtered, crude protein from the *Leuconostoc* cultures after sterilization at 121°C for 15 min was tested on each target bacteria.

Analysis of bile salt tolerance

The effect of bile salt on growth rate of *L. lactis* was evaluated according to the method developed by Walker and Gilliland (1993). The cultures were evaluated for growth in MRS broth supplemented with 0.1, 0.15, 0.20, 0.25 and 0.30% bile salt (Difco, 0129-02), with the acidity adjusted to a pH of 3 using 0.1 N HCl. Fresh cultures of *Le. lactis* were inoculated into each MRS broth containing different bile salt concentrations and were incubated at 37°C in a water bath. The growth was monitored hourly for a period of 11 h using a spectrometer at an optical density (OD) of 620 nm.

Effect of bile salts and pH on the growth rate of L. lactis

The growth rate of *L. lactis* in tubes containing 10 mL of MRS with different concentrations of bile salts (0.1, 0.2, and 0.3%) and the following two pH conditions were determined: one set of tubes was adjusted to pH 3.0 and the other was adjusted to pH 6.0 with 0.1 N of HCl. All the solutions were autoclaved at 121°C for 15 min, cooled and then inoculated with 100 µL of fresh cultures that had been grown overnight. The MRS inoculations were then incubated at 37°C for 24, 48 and 72 h. Each treatment was tested in triplicate. The turbidity, as an indication of growth or no growth, was monitored hourly at an OD of 620 nm using a spectrophotometer.

Effect of Tween 80 (1%) on the tolerance of *L. lactis* at different concentrations of bile salts

Different concentrations of bile salts (0.1, 0.2 and 0.3%) were added to test tubes of MRS broth containing 1% Tween 80 (v/v). The pH was adjusted to 3, and the tubes were then autoclaved at 121°C for 15 min. Each tube was inoculated with 100 µL of broth cultured overnight and incubated at 37°C for 6, 12, 24, 48 and 72 h. The bacterial growth rate was determined at an OD of 620 nm using a spectrophotometer.

Autolysis of whole cells in buffer solution

L. lactis cells in the exponential phase of growth (OD at 620 nm = 1±1.5) were centrifuged at 3000 *g* and 4°C for 10 min. The cells were harvested and washed in potassium phosphate buffer (50 mM at pH 6.5). The cells were then suspended in the same buffer to an OD at 620 nm of 0.6±0.8 and incubated at 30°C. The percent decrease in the OD of the cells was expressed as the extent of autolysis after 7 h of incubation in a water bath at 30°C: lysis (%) = 100 - (A1/A2) x 100, where A1 is the lowest OD value and A2 is the maximal OD value measured during the incubation period (Cibik, 2010).

Preparation of cell extracts and polyacrylamide gel electrophoresis (PAGE)

Cell extracts from bacterial cultures of *L. lactis* were prepared after 24 h of growth in fresh liquid MRS medium, according to the method described by Yehia and Al-Dagal (2014). One hundred microliters of the overnight cultures were inoculated into 10 mL of fresh liquid MRS medium, and the cells were then grown for 3 to 4 h until they reached an OD at 620 nm of 0.6. The cells were collected, weighed (250 mg) and then suspended in 100 µL of TES buffer (50 mM Tris, 1 mM EDTA, and 25% sucrose, pH 8). Mutanolysin (5000 IU/mL) and 20 µL of lysozyme (50 mg/mL) were added to the suspension cells in TES buffer, and the cells were then incubated at 37°C for 30 min. Ten microliters of 20% SDS was added, and the contents were mixed until the solution was clear. Forty microliters of total protein extract was loaded onto SDS-PAGE gels. The SDS-PAGE of the isolates and the running of the samples were performed using a 12% polyacrylamide running gel and a 4% stacking gel with a 0.025 M Tris/0.19 M glycine buffer at pH 8.3 and 100 µL of a sucrose buffer (50 mM Tris-HCl, pH 8; 40 mM EDTA, pH 8; 0.75 M sucrose). Using a vertical tank apparatus, electrophoresis was performed at 25°C with a constant voltage power supply until the bromophenol blue tracking dye reached the bottom of the gel. The gels were stained with 0.25% Coomassie Brilliant Blue R-250 (Bio-Rad, Marnesla-Coquette, France) in a ratio of water:methanol:acetic acid 6.5:2.5:1 for 18 h at room temperature. Gel destaining was performed by continuous agitation in a ratio of methanol:acetic acid:water 20:10:70 v:v:v solvent until obvious protein bands were obtained.

Renaturing SDS-PAGE (zymogram)

SDS-PAGE electrophoresis renaturation was performed as described by Potvin et al. (1988) and Lepeuple et al. (1998b) with 12.5% (w/v) polyacrylamide separating gels in a Mini-Protean I cell. Autoclaved cells (120°C for 15 min) of *L. monocytogenes*, *B. cereus*, *B. subtilis, S. aureus* and *M. luteus* were incorporated separately into polyacrylamide gels at a concentration of 0.2 to 0.4% (w/v) and used to test the bacteriolytic activity of *L. lactis*. The polyacrylamide gels were soaked in 250 mL of distilled water for 30 min at room temperature under gentle agitation. The gels were then transferred to 200 mL of renaturing buffer (50 mM of 1X potassium phosphate buffer at pH 6.5 with 0.1% Triton X-100) and incubated at 37°C with gentle agitation for 16 h. The gels were stained in 0.01% KOH containing 0.1% methylene blue for 2 h and destained in distilled water. Clear zones of lytic bands appeared in the opaque background. The molecular weights of the lytic bands were determined in comparison with standards run on the same gel.

Statistical analysis

Experiments were replicated twice (n=2) on different occasions with

Table 1. Identification of bacterial species based on API 50 CHL kits and its percentage (%)

Bacterial isolates from chicken carcass	Number of strains	Percentage (%)
Lactobacillus delbrueckii	1	11.11
Leuconostoc lactis	3	33.33
Lactobacillus salivarius	2	22.22
Lactobacillus acidophilus	1	11.11
Lactobacillus fermentum	1	11.11
Lactobacillus brevis	1	11.11

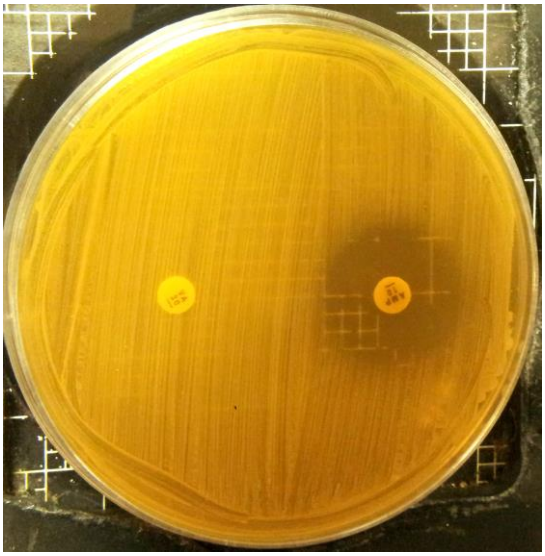

Figure 1. Figure 1. Vancomycin-resistant *Leuconostoc lactis* and sensitive to ampicillin.

different chicken samples (total number of chicken samples = 60). In each of the two experiments, 30 chicken samples were analysed in triplicate for each replicate. Results are reported as mean values ± standard deviation (S. D.). Data were analysed using the software Stat graphics (Statistical Graphics Corp., Rockville, MD, USA).

RESULTS

The results presented in Table 1 show the types of bacterial isolates isolated from chicken carcasses. According to the API 50 CHL assays, these isolates belonged to five different species of the genus Lactobacillus and one isolate belonged to the genus, Leuconostoc. The percentage of each species identified, out of the total number of species examined, varied as follows: *L. lactis* presented the highest ratio (33.33%), followed by *Lactobacillus salivarius* (22.22%), and the lowest ratios were obtained for *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Lactobacillus brevis* (each at 11.11%).

Figure 1 depicts an example of *L. lactis* species resistant to 30 µg of vancomycin, and sensitive to ampicillin 10 µg, as determined through the disc diffusion method with Mueller-Hinton agar. The additional identification of this strain as catalase-negative was detected by adding one drop of H_2O_2. Figure 2 shows effect of the whole crude protein extracted of *L. lactis* cells against the target bacterial species, *B. subtilis*, *B. cereus* and *L. monocytogenes.* Pronounced inhibition zones were observed for *Leuconostoc* cells against all three of these bacteria. The addition of methylene blue clarified these zones. Zhang et al. (2013) reported that *L. lactis* isolated from the intestine of black porgy fish was most effective against the growth of *Escherichia coli* strain O157:H7, *Salmonella typhimurium*, *B. subtilis*, *Proteus vulgaris*, *Vibrio parahaemolyticus*, *Vibrio*

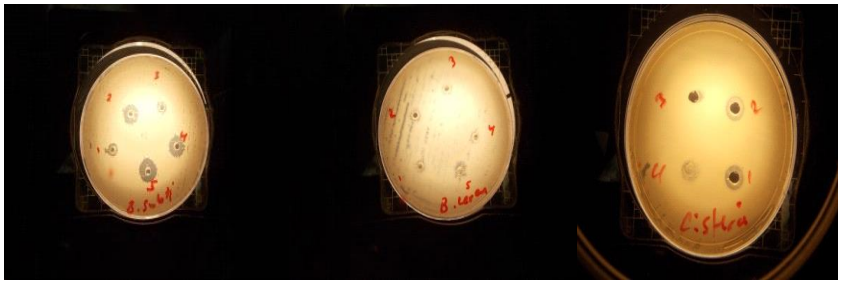

Figure 2. Detection of the inhibitory effects of whole crude proteins extracted from cells (50 µL) of *L. lactis* against *Bacillus subtilis*, *Bacillus cereus* and *Listeria monocytogenes*. The inhibitory effects were determined through the well diffusion method.

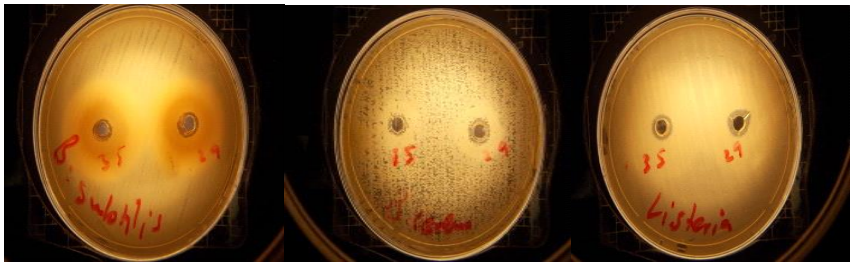

Figure 3. Detection of inhibitory effects of whole crude proteins extracted from cells (50 µL) of *L. lactis* and sterilized at 121°C for 15 min against *B. subtilis*, *B. cereus* and *L. monocytogenes*. The inhibitory effects were determined through the well diffusion.

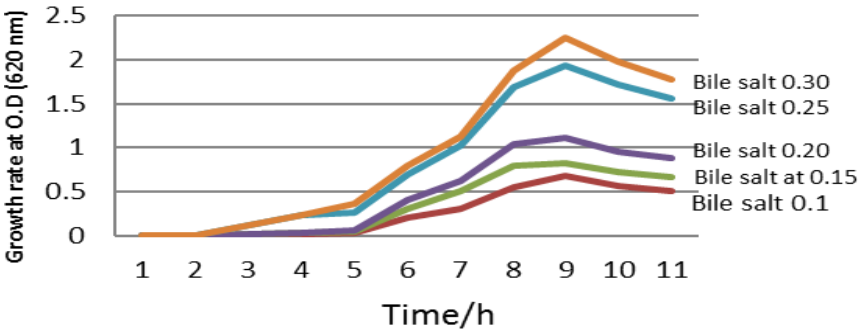

Figure 4. Growth of *L. lactis* in MRS broth after 11 h of cultivation in the presence of bile salts.

alginolyticus, *Vibrio harveyi* and *Shigella,* but showed low inhibition towards *S. aureus* and no inhibition towards *L. monocytogenes*, *L. lactis subsp. cremoris* and *Aspergillus niger*.

The most interesting finding in the present study is shown in Figure 3, which shows that the crude protein extracts of the cells were also effective against the three tested bacteria after sterilization at 121°C for 15 min. Tolerance to bile salts allows bacterial strains to live in the intestines of hosts and to fulfil their metabolic activity while tolerating such harsh conditions. Thus, the ability of *L. lactis* to grow in the presence of different concentrations of bile salts (0.1, 0.15, 0.20, 0.25 and 0.30%) was studied individually and in conjunction with varying pH values. Figure 4 shows that the growth rate of *L. lactis* reached its maximum at a bile salt concentration of 0.3% after 9 h of incubation at 37°C (OD of approximately 2.5). The degree of tolerance of *L. lactis*

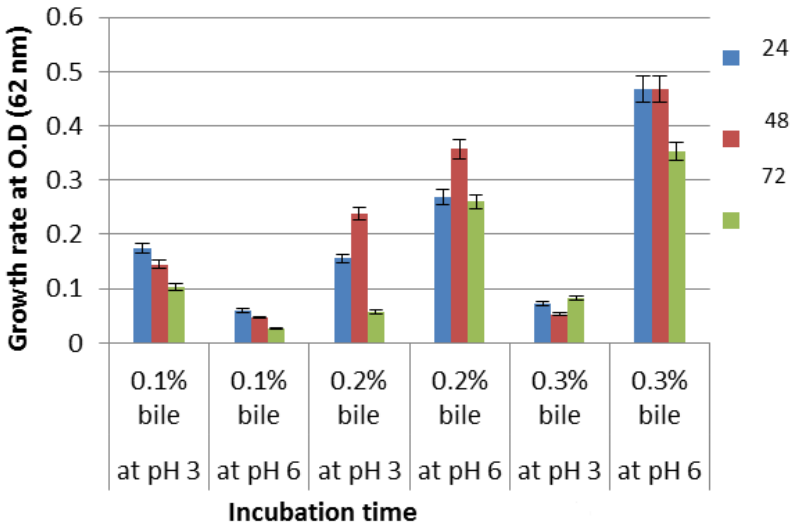

Figure 5. Growth of *L. lactis* in MRS broth after 24, 48 and 72 h of cultivation in the presence of different concentrations of bile salts and at different pH values.

against bile salts was quite apparent. Resistance to bile salt of the isolates could be attributed to their ability to produce bile hydrolase(Savage 1992).Bile salt hydrolase (BSH) protects the cells that produce it from the toxicity of conjugated bile salts by deconjugating the bile acids (Walker and Gilliland, 1993).

Figure 5 shows the effect of different concentrations of bile salts (0.1, 0.2, and 0.3%) and different pH values (3 and 6) on growth rates. The maximum growth of *L. lactis* was obtained at the bile salt concentration of 0.3% and a pH value of 6, with an OD value greater than 0.45 after 24 and 48 h and an OD value greater than 0.35 after 72 h. The results presented in Table 2 show that the growth of *L. lactis* in the presence of bile salt concentrations of 0.1 and 0.2% was better than that observed at a bile salt concentration of 0.3% and allowed the bacteria to reach their maximum growth (OD: 6.5) after 48 h. Increasing the bile salt concentration to 0.3% reduced the growth rate to an OD of 2.0 within the same incubation period. Although, *L. lactis* grew well in the presence of bile salts in the absence of Tween 80, the growth in the presence of 1% Tween 80 was enhanced. At 72 h, a high decrease in the growth of bacterial strain was noted probably due to the depletion of most nutrients of the media, and the stress that the bacterial strain was exposed to, during growth in the presence of bile salt and Tween 80.

Figure 6 shows a reduction in the growth phase and autolysis of the bacterial strain *L. lactis* at the end of the exponential phase. Lysis at 18% was obtained after 7 h of incubation, suggesting that the strain of *L. lactis* was partially autolysed. Figure 7A shows the Coomassie blue staining of the total protein extracted from *L. lactis*. A zymogram analysis of the lytic activities of whole-cell extracts of *L. lactis* using *B. cereus, B. subtilis and L. monocytogenes* as substrates, was performed using renaturing SDS-PAGE (Figures 7B to D). Two bacteriolytic activity bands were revealed after 2 h of incubation in renaturation buffer at 37°C. The two major bands had apparent molecular masses of approximately 57 and 42 kDa. The expression of genes encoding the autolysin of the two bands that resulted in the lytic zone was observed on the gels. No bands were detected for *M. luteus* or *S. aureus*. A band at 14 kDa revealed another lytic band referred to as the lysozyme, which was used for the degradation of bacterial cells.

DISCUSSION

The results, regarding *Lactobacillus* species isolated from chicken carcasses in Saudi Arabia, are similar to those reported in other countries. For example, Ibourahema et al. (2008) isolated *L. casei*, *Lactococcus lactis*, *L. plantarum* and *L. paraplantarum* from poultry farms in Senegal. In Nigeria, Adesokan et al. (2008) isolated three different LAB species from poultry meat and they reported that *L. plantarum* represented the highest percentage of total species at 90%, whereas *L. mesenteroides* and *L. brevis* each represented 5% of the total bacterial populations. In Indonesia, Lengkey et al. (2009), isolated *L. lactis* ssp. *lactis* 1 and *lactis* 2, *L. fermentum* 1, *Lactobacillus paracasei* 1 and *Lactobacillus rhamnosus* from raw poultry meat. Lin et al. (2007) showed that *L. fermentum* is the major LAB species found in the gastrointestinal tracts of swine and poultry.

Table 2. Effect of Tween 80 (1%) on tolerance to bile salt.

Bile salt concentration (%) / O.D at 620 nm	0.1					0.2					0.3				
	Time (h)					Time (h)					Time (h)				
	6	12	24	48	72	6	12	24	48	72	6	12	24	48	72
Bile salt + 1% Tween 80	0.5	1.1	2.0	0.65*10	2.0	0.5	1.3	2.0	0.65*10	2.0	0.6	0.9	1.8	2.0	2.0
Bile salt without Tween 80	0.2	0.8	1.5	0.6*10	0.9	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3

All the above-mentioned authors used API CHL 50 kits for the identification of LAB species in poultry meat. Zhang et al. (2013) stated that *L. lactis* isolated from the intestine of black porgy fish is most effective towards *E. coli* O157, *S. typhimurium*, *B. subtilis*, *P. vulgaris*, *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi* and *Shigella*, whereas *L. lactis* displayed a lower inhibitory activity towards *S. aureus* and did not show any inhibition on *L. monocytogenes*, *L. lactis* subsp. *cremoris* and *A. niger*. The protein produced by *L. lactis* was found to be thermostable at high temperatures, which may be of great importance for its action against microorganisms in food treated at high temperatures during the preservation process.

Due to the presence of bile salts and low pH in the intestine, the evaluation of LAB as probiotics depends on their ability to tolerate these conditions (Fontana et al., 2013). It is also important for probiotic bacteria to be able to grow in 0.15±0.30% oxgall (Goldin and Gorbach 1992).

Kimoto et al. (2002) showed that the addition of Tween 80 (a non-ionic detergent) to the microbial medium containing bile salt enhances the microbe's tolerance to bile, although the effect of Tween 80 was influenced by the type of strain and bile constituents. Kimoto et al. (2002) also investigated the effect of adding Tween 80 on cellular permeability, and the results revealed that the addition of Tween 80 in the presence of bile reduced the cellular leakage (caused by 0.3% oxgall). Cellular leakage was caused by oxgall, whereas cell lysis was not enhanced by oxgall. Therefore, the leakage of UV-absorbing materials is due to an increase in cellular permeability and not cell lysis. These results indicate that exogenously added Tween 80 reduces the cellular permeability caused by bile. Starvation conditions are obtained by transferring bacterial cells from the culture medium to a buffer system, and this technique is widely used to estimate the potential autolytic properties of bacteria (Cibik and Chapot-Chartier, 2004; Lortal et al., 1989; Lemee et al., 1994). Similar results have been reported for *L. lactis lactis* (Mou et al., 1976; Ostlie et al., 1995a). Buist et al. (1995) reported that inactivation of the *L. lactis* gene encoding the major autolysin AcmA results in the detection of several bands by renaturing gel electrophoresis that correspond to degradation products or to a precursor form of AcmA. A similar situation occurs in *S. aureus*, in which most of the multiple bands detected by renaturing SDS-PAGE result from the processing of the major autolysin Atl (Foster, 1995). In agreement with previous reports (Ostlie et al., 1995b; Chapot-Chartier 1996; Lortal et al., 1997), this study revealed that the peptidoglycan hydrolase profile of strains within a species is conserved.

However, in contrast to previous observations for the Lactobacillus genus (Lortal et al., 1997), the peptidoglycan hydrolase profile does not appear to be specific to one species in the Leuconostoc genus but may rather be an indication of species relatedness. Ostlie et al. (1995a, b) and mentioned that cells express the highest level of autolysis in Propionibacterium and *Lactobacillus acidophilus* during the exponential phase of growth, which supports the hypothesis of the involvement of autolysins in cell growth (Crow et al., 1995). In contrast, Buist et al. (1995) postulated that a major autolytic enzyme from lactococcal strains is expressed during the stationary phase. Alternatively, maximal autolysis for *Streptococcus thermophiles* has been observed in the transitional phase between the exponential and stationary phases (Sandholm, and Sarimo, 1981). Additionally, Valence and Lortal (1995) reported two optimal harvesting points for *L. helveticus*: the first occurs during the transitional phase, and the second occurs during the early part of the exponential phase.

Conclusions

In this study, the lactic acid bacterium *L. lactis* isolated from chickens was found to display a tolerance to 0.3% bile salts at pH 6, suggesting that this bacterium is well-adapted to colonize and survive in the harsh environment of the intestinal tract. Its inhibitory activity on the growth of certain Gram-positive foodborne pathogenic bacteria such as *L. monocytogenes*, *B. cereus* and *B. subtilis,* enhances its importance and contributes to its potentially probiotic properties, which may

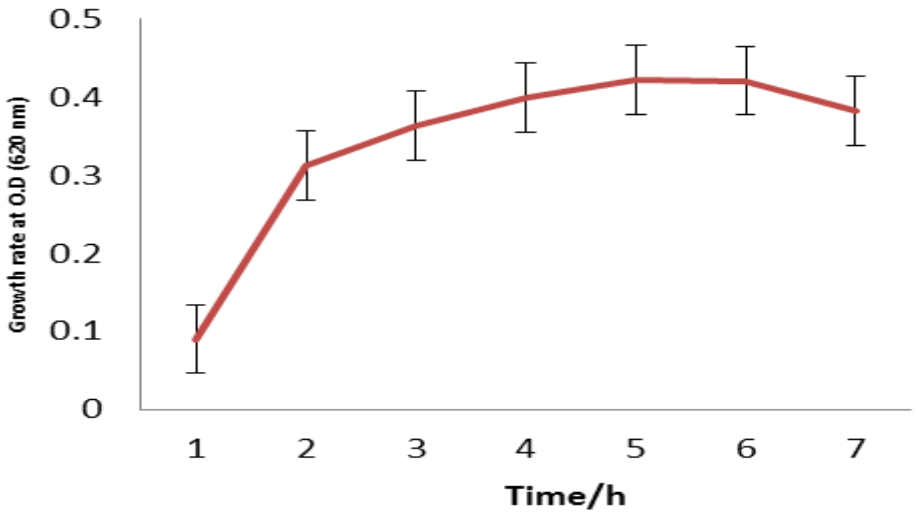

Figure 6. Lysis during incubation of *L. lactis.*

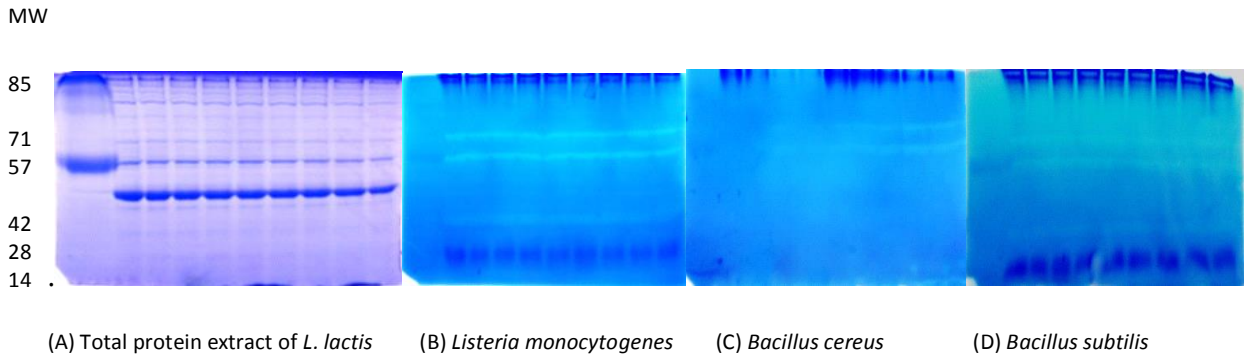

Figure 7. Total protein extracts of *L. lactis* on SDS-PAGE stained with Coomassie blue (A), zymogram analysis of hydrolytic activity against *L. monocytogenes* (B), *B. cereus* (C) and *B. subtilis* (D). Clear lytic bands were visualized for the three tested microorganisms at MWs of 57 and 42 kDa by staining the gels with 0.01% methylene blue and destaining with Milli-Q water.

be of benefit in food preservation technologies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Abbreviation: API, Analytical profile index; **SDS-PAGE,** sodium dodecyl sulphate-polyacrylamide gel electrophoresis; **kDa,** kilo Dalton; **OD,** optical density; **IU,** international Unit; **EDTA,** ethylenediaminetetraacetic acid; **TES buffer,** Tris EDTA buffer; **UV,** ultra violet.

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Full Length Research Paper

Microbiological assessment and hazardous effect of ready-to-eat foods presented for sale in Lucknow City, India

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Street foods play an important role in people's daily food options and their regular nutritional requirements are dependent on these foods, as their ever-growing busy schedule take away the opportunity to eat homemade food. Over the years, many food-borne diseases were reported due to contaminated non-homemade food consumption. This study was conducted to analyze the microbiological quality of foods which are sold on street side. Five most commonly consumed food items (samosa, chole, Panipuri, sandwich and momos) of street side carts of Lucknow City were tested. *Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus* like pathogenic organisms were found to be present in the five food items studied. Aantibiotic sensitivity test was carried out and *E. coli* was found to be resistant to Itranadozole and Rifampicin. *Klebsiella* was found to be resistant to Rifampicin. *S. aureus* was found to be sensitive to all the antibiotics tested (Rifampicin, Vancomycin, Tetracycline and Streptomycin). The study further highlights the level of microbial load found in various available street foods. The microbial load was found to be highest in sandwich then panipuri followed by momos and comparatively less in chole and samosa.

Key words: Microbiological, assessment, hazardous, ready-to-eat food.

INTRODUCTION

Ready-to-eat foods are foods that can be bought directly from street vendors or hawkers or at local markets and eaten immediately without necessarily having to cook before consumption as they have been already prepared by the vendors. Street foods are defined as "ready to eat foods and beverages prepared and or sold by vendors and hawkers especially in street and other similar public places" (FAO, 1987).These are very popular worldwide

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and provide readily available delicacies at a cheaper rate. However, the unhygienic conditions in which these foods are prepared, stored and served raise a question regarding their microbiological quality. These foods can endanger public health by causing various acute and chronic food borne diseases through pathogenic microbes or toxic substances present in them. Despite the economic and nutritional benefits of street foods, the consumption of these roadside foods has been suggested to potentially increase the risk of food borne diseases as street foods are readily contaminated from different sources (Tambekar et al., 2008). In fact, street foods have often been associated with travellers' diarrhea and other foodborne diseases. The presence of *Escherichia coli*, *Shigella dysenteriae, Streptococcus* sp, *Klebsiella,* and *Enterobacter* suggested fecal contamination. Although some *E. coli* are harmless, Enterohaemorrhagic *E. coli* (EHEC) are capable of producing one or more toxin and a particular serotype O157:H7 have been associated with haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopaenic purpura. Also Enterotoxigenic *E. coli* (ETEC) is associated with traveler's diarrhea. Similarly, *Shigella dysenteriae* have been associated with severe bacillary dysenteriae, while *Streptococcus* sp, have been frequently associated with acute sore throat (Adams and Moss, 2008).

During the last few decades, the street food sector has expanded rapidly in urban areas of low and middle-income groups, both in terms of providing access to a diversity of inexpensive foods for low- income households and in offering job opportunities for many urban residents. The street food sector also contributes to the economy of an urban and pre-urban agricultural sector. In India, the National Policy for Urban Street Vendors/Hawkers stated that street vendors constitute approximately 2% of the population of a metropolis (Bhowmik, 2005). Due to its low cost and convenience, an estimated 2.5 billion people worldwide consume street food each day. Most of the studies done on street foods in India and abroad had indicated that these foods are not meeting the microbiological standards and are contaminated with various pathogens viz. *E. coli, Vibrio, Salmonella, Listeria* etc. (Chiou et al., 1996; Ryu et al., 1998; Mosupye and von Holy, 1999; Fang et al., 2003; Lewis et al., 2006). Sandeep et.al, 2005 reviewed the food borne illnesses associated with the consumption of street foods. These food borne illnesses were leading cause of morbidity and mortality worldwide (Bryan, 1988). The microbiological status of the food has been reported to be dependent on several factors like quality of raw material (Jones et al., 1991; Thunberg et al., 2002), handling and processing of food (Jones et al., 1991), microorganisms that survive the preservation and storage treatment (Gimenez and Salgaard 2004), and post process contamination (Long et al., 2002; Meena et al., 2004). Beside direct health consequences, these food borne illnesses can reduce the productivity and economic output, and also impose substantial stress on health care system (Martins and Anelich, 2000; Mosupye and von Holy, 2000). Identification of precise sources of microbial contamination is crucial when devising strategies to reduce further outbreaks. The present study was therefore undertaken to evaluate the microbial quality and safety of consumption of different street foods sold in Lucknow Markets. The objectives of this study therefore are to isolate and characterize some bacterial pathogens from street vended food and to evaluate the antibiotic susceptibility profile.

MATERIALS AND METHODS

Five samples of street foods collected during February to April from different places of Lucknow City are as follows:

1. Panipuri from Utrathia Market
2. Chhole from Hazratganj market
3. Sandwich from BBAU canteen
4. Samosa from CharbaghLucknow
5. Momos from Rajnikhand market

Sample collection

The five street food samples were collected in the month of February and March 2016. Samples were collected in sterile bags for solid and semi-solid foods and in bottles for liquid food items from the market. It was carried with the consumer survey in which 100 subjects were taken regarding their commonly consumed street food. Five locations in Lucknow were chosen for the collection of samples, where the sale was maximum per day. Food samples (samosa, panipuri, sandwich, vegetarian momos and chole) were collected twice from local street shops and franchise's outlets at one month interval. All the samples were aseptically collected in sterile containers, stored at 4°C and analyzed within an hour of collection.

Isolation and enumeration of microorganisms

All the samples were aseptically collected in sterile containers, stored at 4°C. Ten gram of sample was weighted under aseptic condition and properly homogenized by using a sterile pestle and mortar. Ten gram of homogenated sample was added to 90 ml of sterile 0.85% saline water in a test tube and diluted serially upto 10^{-5} dilution was obtained. For bacterial isolation 0.1 ml of dilution from each tube was aseptically pipette out and plate onto different nutrient agar media using spread plate technique. The plating was done in the laminar flow to maintain aseptic conditions. All the plates were placed in an incubator at 37°C for 24 to 48 h in an inverted position. For bacterial enumeration the plates were used to determine the number of colony forming units (CFU) per gram of food.

Nutrient agar, Mac-conkey agar, EMB agar, Manitol salt agar and *Salmonella-Shigella* agar were inoculated for total aerobic plate counts (total heterotrophic counts, coliform bacterial counts and total *Salmonella shigella* counts, respectively. Enumeration of bacteria and isolation of bacterial colonies was done after incubation

Table 1. Microbial load of different street foods.

Sample	Dilution factor	No. of colonies	CFU/ml
Panipuri	10^{-1}	112	1.12×10^{3}
Sandwich	10^{-1}	206	2.06×10^{3}
Samosa	10^{-1}	28	2.8×10^{2}
Momos	10^{-1}	37	3.7×10^{2}
Chola	10-1	35	3.5×10^{2}

Table 2. Organisms isolated from different street food.

Sandwich	*Klebsiella*
Panipuri	*E. coli*
Momos	*Staphylococcus aureus*
Chola	*Pseudomonas aeruginosa*
Samosa	*Klebsiella*

of plates at 37°C for 24 h to obtain viable bacterial colonies. Plates containing 30 to 300 colonies were selected and counted at the expiration of the incubation period using the colony counter (Gallenkamp, England). Bacterial counts were expressed as colony - forming units per gram of food sample (cfu/g).

Morphological identification of isolates

The identification of distinct bacterial colonies was based on standard methods (Cowan and Steel, 1985; Speck, 1976). The bacteria isolated were gram stained and specific biochemical tests were performed. The morphological characterization of each of the isolated colonies was done by observing their shape, colour, texture and appearance. The following biochemical test were performed on the isolates; sugars fermentation, catalase activity, oxidase test, methyl red, citrate utilization, coagulase activity and motility test (Buchansa and Gibbons, 1994).

Antibiotic sensitivity test

A single colony of the purified isolates was inoculated in 5 ml sterile peptone water and incubated at 37°C overnight. Then a loopfull culture was diluted in 5 ml sterile phosphate buffered saline and seeded into Muller Hinton agar. Antibiotic disc (Hi-Media) was placed on the surface of agar and incubated overnight at 37°C. Zone of inhibition was recorded. A control sensitive culture was included in the experiment. The test determines the susceptibility of a microbial species against different antibiotic agents (Baur, 1966; Acharya, 2012).

RESULTS AND DISCUSSION

Microbiological analysis

The result depicted in Table 1 shows that the microbial load was highest in sandwich then panipuri followed by momos and comparatively less in chole and samosa because the samosa and chola gets the heat treatment and spices are added which have an antimicrobial property which reduces the microbial load. To prevent the occurrence of foodborne illnesses, it is important to ensure that foods sold are safe and hygienic. Total plate count was used to measure the general bacteria load of the food sampled and is useful tool in monitoring food process and the results may reflect the hygienic level of food handling and retail storage (Collins et al., 1989). Most of the studies done on street foods in India and abroad had indicated that these foods are not meeting the microbiological standards and are contaminated with various pathogens viz. *E. coli, Vibrio, Salmonella, Listeria* etc. The microbiological status of the food has been reported to be dependent on several factors like quality of raw material (Jones et al., 1991; Thunberg et al., 2002), handling and processing of food (Jones et al., 1991), microorganisms that survive the preservation and storage treatment (Gimenez and Salgaard 2004), and post process contamination (Long et al., 2002; Meena et al., 2004).

Gram negative rods and Gram positive cooci's were present in significant numbers in five street foods (panipuri, chhole, sandwich, samosa and momos). A total of eleven (5) organisms were isolated from street food; *E. coli* was isolated from panipuri, *P. aeruginosa* was isolated from chole, *Klebsiella* was isolated from Sandwich and Samosa and *S. aureus* was isolated from momos (Table 2). The isolates were differentiated on the basis of the cultural and cellular morphological studies, after which they were subjected to various biochemical and physiological test and the isolates were identified. Coliform bacteria are mainly found in water, soil and feacal matter. They are widely distributed in water, soil and vegetation (Rompre et al., 2002). The presence of coliforms in ready to eat food such as vegetable salad, packaged fried rice and egg burger depicts a deplorable state of hygiene and sanitary practices employed during the preparation and packaging of these street foods (Jay, 2005). Coliforms are indication of unsanitary conditions, unhygienic practices during and after production and poor source of water used (Beuchat, 1995). Muinde and Kuria (2005) reported that water used for preparation of street

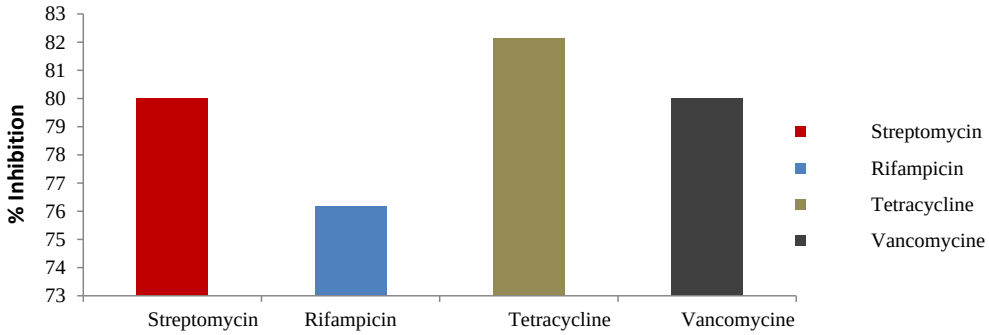

Figure 1. %Inhibition zone against *E. coli.*

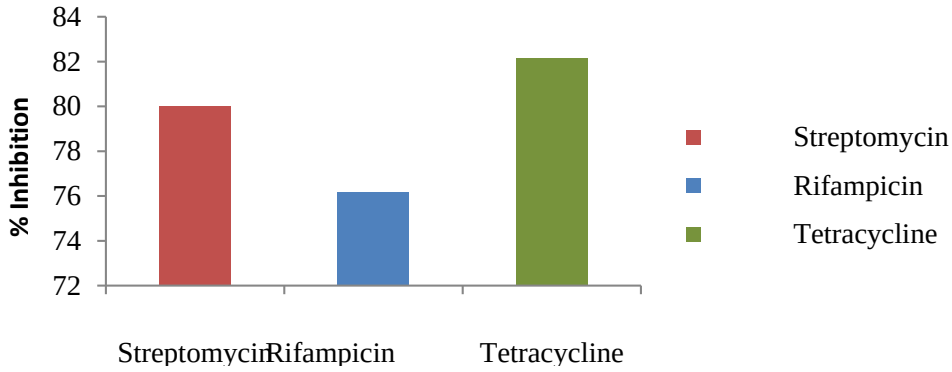

Figure 2. %Inhibition zone of *P. aeruginosa*.

foods is often from sources that are not treated and lead to high bacterial count. The vegetables used in the preparation of the vegetable salad and fried rice always have contact with soil and if not properly washed with clean water could pose a high risk for street food consumers.

The presence of *E. coli*, *Shigella dysenteriae*, *Streptococcus* sp*, Klebsiella*, and *Enterobacter* represent fecal contamination. Some *E. coli* are harmless, however Enterotoxigenic *E. coli* (ETEC) is associated with traveler's diarrhea. Similarly, *S. dysenteriae* have been associated with severe bacillary dysenteriae, while *Streptococcus* sp, have been frequently associated with acute sore throat (Adams and Moss, 2008). Due to washing of vegetables with contaminated water, it gets contaminated with *Salmonella* spp. Also pathogenic microorganisms are scatter through vegetables handling by infected workers, vendors and consumers in the market place. Hazard Analysis of Critical Control Point system (HACCP) study revealed that raw vegetables themselves carried pathogens and since they were not washed they continued to be present at the time of consumption. Sabbithi et al. (2014). Rather, the vendors should be sensitized and also issued some certificates so that they could be trusted and allowed to operate their business.

Antibiotic susceptibility of all the isolated organisms determined by agar diffusion method

E. coli was sensitive (S) to Chloramphenicol and Streptomycin and Resistant (R) to Itranadozole and Rifampicin and Zone of Inhibition of Chloramphenicol was higher than Streptomycin (Figure 1). In case *of P. aeruginosa*, Streptomycin higher Zone of Inhibition was observed as compared with Rifampicin, and Itranadozole was found to be Resistant (Figure 2). *Klebsiella* isolated from sandwich was Sensitive to Streptomycin, Chloramphenicol and Tetracycline but the Zone of Inhibition of Tetracycline was too low as 16.6% and higher Zone of Inhibition was detected in case of Streptomycin as 72%. No zone of inhibition (R) was observed in case of Rifampicin (Figure 3). In case of *Klebsiella* strain isolated from samosa, Streptomycin and Chloramphenicol gave higher Zone of Inhibition than Itranadozole. The strain was found to be Resistant to Rifampicin. The zone of inhibition of Streptomycin,

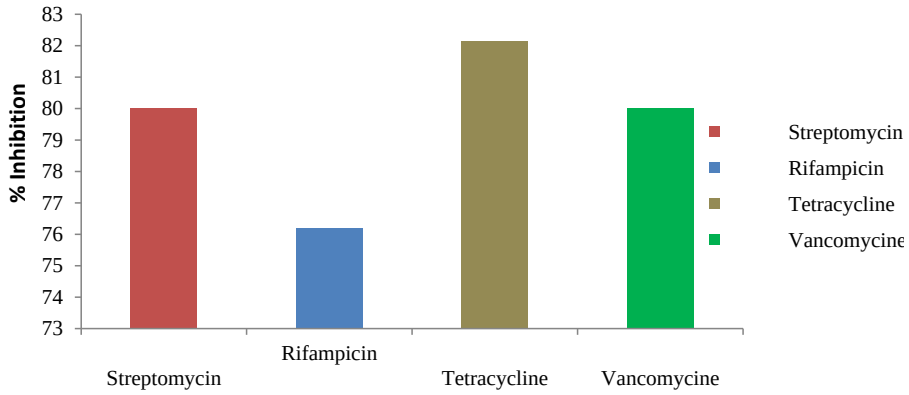

Figure 3. %Inhibition zone of *Klebsiella.*

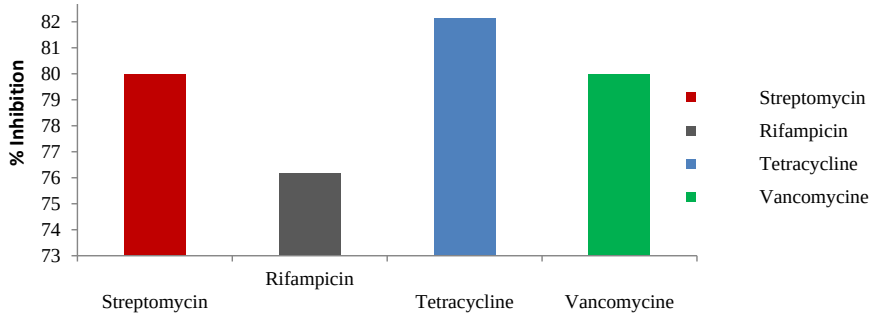

Figure 4. % Inhibition zone of *S. aureus.*

Chloramphenicol and Itranadozole was 68.75, 67.74 and 28%. *S. aureus* was found to be sensitive (S) to Rifampicin, Vancomycin, Tetracycline and Streptomycin (Figure 4). The organisms which were found to be pathogenic*, E. coli, P. aeruginosa, Klebsiella, and S. aureus* were tested for antibiotic sensitivity and these organisms showed resistant to different Antibiotics tested which can have serious health hazard and can pose problem in antibiotic therapy. Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food borne has increased during recent decades (Chui et al., 2002; Davis et al., 1999).The use of antibiotic(s) after the intake of the organism(s) may not be effective as the organisms maybe susceptible or resistant to it. Resistance to antibiotics in food borne pathogens may create problems for disease or illness treatment while antibiotic susceptibility leads to healing of the illness which the organism(s) caused. Traveler's diarrhea is a major inconvenience to visitors arriving in developing Countries from more industrialized areas (Dupont et al., 1982). Earlier studies were also been shown that antibiotic susceptibility results indicated 53.85% resistance and 46.15% sensitivity among vended food isolates. The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades (Boonmar et al., 1998a, b; Chui et al., 2002; Davis et al., 1999; Threfall et al., 2000), possibly as a result of selection pressure created by the use of antimicrobials in food-producing animals (Aarestrup, 1999; Angulo et al., 2000; Bywater, 2004; Teuber, 2001; Van den Bogaard and Stobberingh, 2000). The coexistence of resistance genes with mobile elements such as plasmids, transposons and integrons facilitates the rapid spread of antibiotic resistance genes among bacteria (Sunde, 2005). Also, high rates to antibiotics resistance of bacteria may possibly resulted from inappropriate or uncontrolled use of antibiotics in farming practices, so it is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from antibiotic resistance and pathogenic bacteria originating from food. In addition, the use of antibiotics in animal feeds need to be regulated strongly to minimize the

opportunity for organisms to develop resistance (Van et al., 2007). The result of this study demonstrated that the food samples vended for consumer consumptions were contaminated by pathogenic bacteria which if ingested may be deleterious to consumers' health and may lead to food borne illness or disease.

Conclusion

Gram negative rods and gram positive cooci's were present in significant numbers in five street foods. Hence this study has clearly demonstrated that some of the most popular types of foods (panipuri, chhole, sandwich, samosa and momos) that were vended on the streets of Lucknow City do not meet the required acceptable quality and safety levels. Measures need to be taken to ensure that street vendor food ingredients should be produced and stored hygienically at appropriate temperatures and well protected from flies, dust, wind, and all sources of contamination. Utensils should be washed using detergents and clean hot water. The results of this study have illustrated the extent of antibiotic resistance in all the isolated organisms found. It is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from food borne pathogens; especially those that are originated from street food. Also, strict implementation of food sanitation code and license for street food vendors is needed to make the consumers save. If possible, public health authorities should intensify efforts to monitor conditions of sanitation and hygiene in establishment serving food and drink to the public. So, food safety education is a critical part of the overall strategy to reduce the incidence of food borne illness and complements regulatory and other activities. However, meeting the huge challenge of food safety in the 21st Century will require the application of new methods to identify, monitor and access food borne hazard. Both traditional and new technologies for assuring food safety should be improved and fully exploited. This need to be done through legislative measures where suitable, but much greater reliance on voluntary compliance and education of consumers and professional food handlers. Finally, it is necessary for public health organizations to be concerned since microorganisms causing food borne hazards and food spoilage can be isolated from raw materials and finished products; thus reduction of contamination is an achievable policy objective. It is necessary to pay more attention to food hygiene to reduce or eliminate the risk from food borne pathogens; especially those that are originated from street food. Also, strict implementation of food sanitation code and license for street food vendors is needed to make the consumers save. If possible, public health authorities should intensify efforts to monitor conditions of sanitation and hygiene in establishment serving food to the public.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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