Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Microbiology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the research across the whole spectrum of the subject. These including bacteriology, virology, mycology and parasitology. In addition, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.7 (1)pp. 111-118 (2015)*

Egypt. Acad. J. Biolog. Sci., 7(1): 111-118 (2015)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

Isolation and Identification of Bacteria for Camel's and Goat's Milk. Traditional Dairy of Saudi Arabia

Muneera D. F. ALKahtani
Princess Nourah Bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia
Saudi Arabia P.O. Box 102275 Riyadh 11675
E-mail: mdf.alkahtani@gmail.com

ARTICLE INFO

Article History
Received:3/11/2015
Accepted:15/12/2015

Keywords:
Traditional fermented milk
Identification
pseudomonas putida
Staphylococcus simulanus

ABSTRACT

Camels and goats fermented milk is a traditional product that consumed as a main type of food in nomadic areas of Saudi Arabia, and this bacterium is the predominant microorganism in the camels\sheep milk that is responsible for milk fermentation. In this study, high qualities of fresh milk samples were selected, isolated, and identified total of samples with a fact of 10 fresh milk samples from five different areas in Riyadh City representing North, South, East, and West of Riyadh for analysis. Bacteria have been isolated as follows: *Staphylococcus simulans*, *Erysipelothrix rhusiopathiae*, *Aeromonas Hydrophila*, and *Pseudomonas putida*. In Camels and goats milk, the isolated bacteria were *Staphylococcus* sp bacteria and *Pseudomonas putida* with total of 50% of the study samples, and the isolated bacteria *Kocuria rosea* with 25% and bacteria *Erysipelothrix rhusiopathiae* with 15% and bacteria *Aeromonas Hydrophila* with 10%. Bacteria were identified using a definition of bacteria VITEK 2 and the use of identification systems API 50 CHL API 2C AUX, respectively. The average concentration *psaudomonas* and *staphylococcus* 7.4loglÛ CFU ml7.7loglÛ CFU ml. All isolates of bacteria have been shown in test results and were positive for antibiotics *pseudomonas putida*, *kocuria rosea*, *Erysipelothrix rhusiopathiae, aeromonas hydrophila*, and *Staphylococcus simulant*.

INTRODUCTION

Camel milk and traditional fermented camel milk called shubat are valuable sources of food for people living in steppe and arid areas of central Asia (Faye and Konuspayeva, 2012). These products are widely consumed in Kazakhstan and it is an important part of Kazakh people diet (Konuspayeva and Faye, 2011). Camel milk and shubat microflora plays major fermentative role in the aroma, texture, and acidity; and it has therapeutic role on improvement of digestion properties and responsible for antimicrobials properties (Arab *et al*., 2014). Consumption of fermented milk has many advantages including enhanced nutritional value, digestibility, therapeutic benefits, and safety against pathogens. Traditional camel milk is the most popular fermented milk especially in nomadic herders areas in Saudi Arabia.

Camel and goat milk is prepared from unpasteurized whole sheep, goat, cow, or camel milk. Fresh milk is collected daily in a tanned goats or sheep leather bag container called goat contemning small amount of fermented milk from previous batch as a starter culture. Some herbs called Depagh maybe added together with milk. Then, it is left in the shadow for 1-3 days depending on the ambient temperatures for spontaneous fermentation. The fermented milk may be consumed as such (straight fermented milk) when camel's milk has been used or as in the majority of cases (when used other types of milk), it is churned early in the morning to produce butter. After removing the butter, the sour buttermilk remaining named as "camel milk" is consumed fresh (Saleh, 2010). The main purpose of these processes is to produce butter. The microorganisms, mainly Bacteria (LAB), inherent to this leather bag container (camel and goat milk), and the air in the surrounding environment (Arab *et al*. 2014) are assumed to ferment the milk. The nomads, in general, prefer it due to its excellent natural acidic taste and aroma besides other functional benefits. Also, the people believe in its therapeutic value towards curing or protected from ailments such as diarrhea and constip action as it contains LAB in different species. Moreover, LAB are also reported to colonize the human intestinal mucosa leading to beneficial effects (Fuller, 1992). Saleh (2010) detected some lactic acid bacteria in Sameel milk such as *pseudomonas putida ,kocuria rosea,* and *Staphylococcus simulanus* isolated and identified lusitania and *Cryptococcus laurentii* from traditional fermented milk (Gadag *et al*., 12000) coupled with relatively low counts of yeast. The nature of fermented products varies from one region to another. It depends on the local indigenous microflora, which in turn reflects the climatic conditions of the area (Savadogo *et al*., ،2004). Several investigators from other countries have isolated and identified many lactic acid bacterial species and yeasts from their traditional fermented dairy products. They found that the main LAB genera consisted of *pseudomonas putida, kocuria rosea,* and *Staphylococcus simulanus* (Wangoh, 2005). Enumeration and identification of microflora m Susa fermented camel milk product lacks some scientific information, and not much was found about the traditional fermented milk in Saudi Arabia. In addition, no study has been initiated to identify the fermenting organisms of camel and goat milk to the species level. Thus, the current study aimed at the isolation, identification, and characterization of microorganisms that are responsible for fermentation of camel milk.

MATERIALS AND METHODS

Milk Samples:

Milk is a suitable environment for the growth of food microbiology at the availability of appropriate degrees of heat. It is rich in protein, carbohydrates, fats, vitamins, and minerals which is an important addition to the appropriate function of acidity and moisture activity and is susceptible to damage by bacteria, yeasts, and molds and rapidly. Common races in milk are micrococcus, pseudomonas, and Lactobacillus Streptococcus. Ten samples of camel and goat milk were collected from four different villages of the Riyadh region of Saudi Arabia (three samples of milk camel’s south of Riyadh, two samples of milk camel’s west of Riyadh, three samples of goat's milk south of Riyadh, and two samples of goat’s milk east of Riyadh. The work series starts with dilution of a sample of milk and take 1 ml or 0.1 ml of appropriate mitigation to dishes sterile, then add to the center Manitol salt agar, MacConkey agar, and Nutrient agar, and by two dishes for each dilution and incubated dishes at 37 ° C for 24-48 commodity and longer courses and the number of bacteria and isolate and analyze Dilution.

Determination of pH:

The pH was determined using a Gmson pH meter (GPL21) (Herisau, Switzerland) after calibration using standard

buffers (Metrohm Ion Analysis, Herisau, Switzerland) at pH 4 and 7.

Microbial Enumeration and Isolation:

Ten milliliter from each fermented milk sample was transferred aseptically into 90 ml peptone water solution and mixed thoroughly. Serial dilutions (10^1 -10-8) were performed and 1 ml, aliquot of the appropriate dilution was incubated in triplicate on universal and selective media. Plate count agar (Oxoid CM0325) incubated at 30°C for 72 h for enumeration of total aerobic mesophilic bacteria. MRS agar (Oxoid CM0361) incubated anaerobically at 3C)°C for 48 h for. M 17 agar (Ox-oid CMC) 785) incubated anaerobically at 3C)°C for 48 h for pseudomonas putida, Kanamycm aesculin azide agar (KAA) (Oxoid CMC) ö91) incubated at 37^0C for 48 h for enumeration of. Staphylococcus simulanus Violet red bile agar (VRBA) (Châocl CM 0107) incubated anaerobically at 37^0 C for 24 h for enumeration of, kocuria rosea Acidified potato dextrose agar (PDA) (Oxoid CM0139) was incubated at 30^0 C for 48 h for enumeration of yeasts and moulds, (0.1 ml, of the appropriate dilution spread plated on this medium). The anaerobic condition was performed in anaerobic jars (Biolab) with gas generating kits (Oxiod BROC) 38B). Representative bacterial colomes were isolated randomly from plates of MRS, Ml 7 and KAA agar. Isolates were cultivated in its selected broth medium and incubated at 30^0C for 24 h. The isolates were purified by streak plating using the same medium. The bacterial isolates were re-suspended and stored in its selected medium containing lõû û glycerol at-18° C. Representative yeast colonies on PDA were examined by phase contrast microscopy and purified by successive streaking on PDA. The pure yeast isolates were stored slants at 4° C.

Material Used:

Manitol salt agar, MacConkey agar, Nutrient agar, distilled water, Smpil milk, and vitke system.

Identification of Using the Biomerieux Vitke System:

Vitke automated microbiology system and its application in the identification of microorganisms.

PRINCIPLES

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. Figure 1 shows the VITEK 2 compact system. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

Fig. 1: VITEK 2 compact

RESULTS AND DISCUSSION

Isolation and enumeration of microorganisms in Table 1 show the presence of microbes in the samples of milk from camels and goats from difference parts. *pseudomonas* and *Staphylococcus* were all tested samples with average viable counts of 7.4 and 7.7 loglÛ CFI-J mL-l with a range of '3.3-8.7 and 6.5-8.9 log10 CFU] mL l, respectively, with the corresponding average aerobic mesophilic bacterial counts of 6.6 log] o CFL] mL. Actually, the average counts of LAB (pseudomonas and staphylococcus) of camel milk were similar to that of yoghurt, which normally was 6-8 log CFI_T mL-l (Gou, 2003). Many

potential health or nutritional benefits from some species of LAB were reported as: improved nutritional value of food, control of intestinal infections, improved digestion of lactose, control of some type of cancer, and control of serum cholesterol levels (Gilliland, 1990) lower than either the counts of pseudomonas or staphylococcus by almost two log-cycle.

Table 1: Isolation and identification the bacteria to different Riyadh regions

Milk sample	West Camel 1	South Camel 1	South Camel 2	West Camel 2	South Camel3	North Goat 2	South Goat 2	South Goat 1
Isolate bacteria	*Pseudomonas putida*			*Staphylococcus simulans*	*Aeromonas hydrophila*	*Kocuria rosea*		*Erysipelo thrixrhusi opathiae*
LAB (log10 CFU	6.5-8.9			3.3-8.7	6.5-7.7	6.6-7.7		7.4-7.7

The study of the effect of antibiotics on bacteria obtained: Table 2 and Table 3 the antibiotic is a materials-chemicals membership is made up as the product of the vital activity of some microorganisms and has an impact pesticide or position for the growth and the activity of other micro-organisms, each antibiotic in his own way. Some bacteria works to prevent the formation of the wall like Albesellnat, while others focused on the impact of interference on the vital cell systems and prevent the formation of protein cell bacteria Negative result, +-: Positive result, e: Doubtful result as described in material and methods.

Table 2: The sensitivity of these bacteria to some resistance

Milk sample	**Isolate bacteria**	**sensitive**	**Resistance**
West Camel 1 South Camel 1	*Pseudomonasputida*	*Amikacin* *Cefotaxime* *Ciprofloxacin* *Gentamicin* *Imipenem* *Piperacillin/tazobactam*	*Amoxycillin/Clavulanicacid* *Ampicillin* *Cephalaxin* *Cefuroxim* *Nitrofurantion* *Cephazolin* *Ampicillin/Sulbactam*
West Camel 2	*Staphylococcu ssimulans*	*Ciprofloxacin* *Cefoperazone* *Cefepime* *Ofloxacin*	*Amoxycillin* *augmentin*
South Camel 2	*Pseudomonasputida*	*Cefoperazone* *Cefepime*	*Sulfamethoxazolen/trimethopim* *Amoxycillin*
South Camel3	*AeromonasHydrophila*	*Cefepime* *Nitrofurantion* *Gentamicin* *amikacin*	*Ampicillin* *Ampicillin/sulbactam* *cephazolin*
South Goat 1	*Erysipelothrixrhusiopahiae*	*Augmentin* *Cefoperazone* *ciprofloxacin*	*Amoxicillin* *oxacillin*
South Goat 2	*Kocuriarosea*	*Augmentin* *Cefoperazone* *Penicillin G*	*Oxacillin* *Nitrofurantion*
North Goat 1	*Kocuriarosea*	*Augmentin* *Cefoperazone* *Piperacillin /tazobactam*	*Oxacillin* *Sulfamethoxazolen/trimethopim*

Table 3: The sensitivity of these bacteria to some antibiotics such as test: Cefoxitin (FOX), Vancomycin (VA), Gentamicin (GM), and Nitrofurantion (FlM)

Cefoxitin(FOX)	+
Vancomycin (VA)	-
Gentamicin (GM)	+
Nitrofurantion(FlM)	+
Penicillin G (PG)	-

Table 4 and Table 5 The ratio of the results of the sensitivity test isolates showed a difference in sensitivity isolates and resistance towards life antibiotics and different proportions towards the species used in the study, which included (5) types of life antibiotics and they are: Clavulanic acid/ amoxicillin, Cefoxetine, Ciprofloxacin, Erythromycin, and Ampicillin. The results showed that the sensitivity ratios for each of Ampicillin, Cefoxetine, Clavulanic acid / amoxycillin Methec reached (50%) and was (79%) of the isolates were sensitive to anti-(*Nitrofurantion*). The Anti (Ampicillin) was effective against (57%) of the isolates, while the most efficient antibiotics was (Ciprfloxacin) and has proved effective against isolates since all isolates were sensitive to this counter and reached (100%).

Table 4: Microbial viable counts and ph of camel milk and goat milk from Riyadh villages show samples having low pH values (<4.2).

NO	**Samples**	**pH**	**aerobic mesophilic bacteria**	**staphylococcus**	**Pseudomonas**	**kocuria**
1	Cs1	4.1	6.5	7.8	8.5	7.2
2	Cs2	4.5	5.3	6.0	8.1	6.1
3	Cs3	4.6	7.9	6.8	6.5	6.0
4	Cw4	5.5	7.8	8.2	7.3	4.9
5	Cw5	4.2	6.9	7.6	7.6	3.1
6	Gs1	4.1	7.3	8.5	8.0	5.4
7	Gs2	4.1	3.	7.6	7.8	3.9
8	Gs3	4.1	6.5	8.7	8.7	4.2
9	Gn4	4.4	5.3	6.9	8.3	5.1
10	Gn5	4.5	7.9	6.6	7.0	7.2
	Cs1	4.1	6.5	7.8	8.5	6.1

Table 5: Reductase Dye Reduction (RDR)

Sample milk	camel South 1-2-3	Camel West 1-2	Goat north 1-2	Goat south 1,2,3
Reductase dye	Not reduced during the period of the dye more than eight hours	Reduced pigment within 6-8 hours	Reduced during 2-6 hours	Reduced pigment in less than a two-hour period
The result	Excellent *	* Good	* Center	. bad *

This test is used to determine the biological activity of the bacteria in the milk if commensurate activity directly proportional to the number and rate of respiration by preparing Aldrov non-pneumatic Vtakhtzel tints. Used for this purpose are two types of dyes which are Methylene blue and Resazurin.

To transport (10 ml) of milk into a sterile test tube with the payment of a spiral and added to (1 ml) of Methylene blue dye and mix well the heart tube quietly and make sure there are no gas bubbles do not even oxidized dye.

ETT is incubated in a water bath and licked every half hour for 6 hours. Okellma increased speed reduction, it means milk containing the larger numbers of bacteria (Fig. 2).

1. Excellent: If the dye did not boil over a period of more than eight hours.

2. Good: If the dye reduced during the period of 6-8 hours.
3. Center: if reduced dye during the 2-6 hours.
4. Bad: If the dye reduced during the period of less than two hours, of all staphylococcus in goat milk and camel as staphylococcus simulans. From a total of 120 isolates from fermented milk, Isono *et al*. (1994) identified only 3 strains as staphylococcus. Cueto *et al*. (2007) identified 15 out of 36 LAB isolates as staphylococcus.

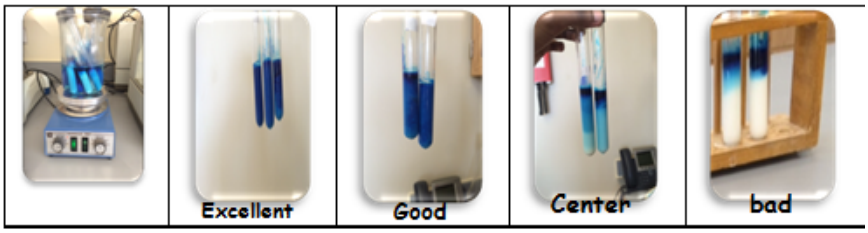

Fig. 2: Reductase Dye Reduction (RDR)

In this study, ten strains were identified as staphylococcus sp. All of these strains are able to grow in MRS at pH 3.9 and m the presence of '3.59/0 NaCl. However, Lactobacillus pentosus has not been previously reported m tractional fermented milk, but it has been isolated from plant materials and fermented food (Osawa *et al*., 2000; Sawitzki *et al*., 2007). Whereas, Cueto *et al*. (2007) identified only one strain of staphylococcus from 36 LAB strain isolated from traditional fermented milk. Thirteen strains were identified as staphylococcus ssp, six as staphylococcus simulans, five as pseudomonas and three as kocuria sp. All of these LAB were frequently found in the various traditional fermented milk in many countries of the world (El-Soda *et al*., 2003; Lore *et al*., 2005; Sulieman *et al*., 2006; Cueto *et al*., 2007; El-Baradei *et al*., 2008; Kayagil and Candan, 2009). A very little is known about its source and role in the goats and cemal milk products which needs further studies.

Some researchers isolated and identified C. lusitania and Cryptococcus laurentii from traditional fermented milk. Gadaga *et al*. (2000) identified 11 strains as C. lusitania and one strain as Cryptococcus /a urentii isolated from Zimbabwean traditional fermented milk. Kebede *et al*. (2007) isolated and identified Cryptococcus laurentii strains. The growth response obtained could be explained by the utilization of the trace amounts of glucose and galactose in milk (Rosenthal, 1991). In other investigation, S. cerevisiae has been isolated from traditional fermented milk (Gadaga *et al*., 2000; Abdelgadir *et al*., 2001; Cosentino *et al*., 2001; Shuangquan *et al*., 2006).

From the isolates, Candida kefyr were identified. Although C. kefyr has a low incidence in the samples but they are known to be important in dairy products (Fleet, 1990; Seiler and Busse, 1990) and their presence in the milk sample could be important. After identifying in camel and goat milk isolation, there is need for investigation of their technological properties to select the most appropriate strains as starter culture for a controlled and optimized process. In reality, some strains of Lactobacillus paracasei ssp paracasei are probiotic culture (Tharmaraj and Shah, 2004; Theger *et al*., 2009). However, the presence of Lactobacillus paracasei ssp. paracasei in camel milk increases its therapeutic value. Isolation of some strains of this species will provide an opportunity for further investigation.

REFERENCES

Abdelgadir, W.S., S.H. Hamad, PL. Moller and M. Jakobsen, (2001). Characterisation of the dominant microbiota of Sudanese fermented milk rob. Int. Dairy J. 11: [i]33-70.

Arab HH, Salama SA, Eid AH, Omar HA, El-Shaimaa AA, Maghrabi IA (2014). Camel's milk ameliorates TNBS-induced colitis in rats via down-regulation of inflammatory cytokines and oxidative stress. Food Chem. Toxicol., 69: 294-302.

Cueto, C., D. Garcia, F. Garces and J. Cruz, (2007). Prelimmary studies the microbiological characterization of lactic acid bacteria in Suero costeno

El-Baradei, G., A. Delacroix-Buchet and J.C. Ogier, (2008). Bacterial biodiversity of traditional Zabady fermented milk. Int. J. Food Mcrobiol., 121: 295-301.

Fleet, G.H. (1990). Yeasts in dairy products. J. Applied. Bactemol., 38: 199-211.

Faye B, Konuspayeva G (2012). The sustainability challenge of the dairy sector- The growing importance of the non-cattle milk production worldwide. Int. Dairy J. 24:50-56

Gadaga, T.H., A.N. Mutukumira and J.A. Narvhus, (2000). Enumeration and identification of yeasts isolated from typing traditional fermented milk. Int. Dairy J., 10: 459-466.

Jakobsen, M. and J. Narvhus, (1996). Yeasts and their possible beneficial and negative effects on the quality of dairy products. Int. Dairy J., 60: 755-768.

Harrigan, W.F. and M.E. McCance, (1986). Laboratory Methods in Food and Dairy Microbiology.

Lore, T A. SK. Mbugua and J. Wangoh, (2005). Enumeration and identification of microflora m suusac, a Kenyan traditional fermented camel milk product. Food Sci. Technol., 38: 125-130.

Mathara, J.M., U. Schillinger, P.M. Kutima, SK. Mbuguav and W.H. Holzapfel, (2004). Isolation, identification and characterisation of the dominant microorganisms of kule naoto: The IMaasa1 traditional fermented milk in Kenya. Int. J. Food Mcrobiol., 94: 269-278.

Mathara, J.M., U. Schillinger, P.M. Kutima, SK. Mbuguav and W.H. Holzapfel, (2004). Isolation, identification and characterisation of the dominant microorganisms of kule naoto: The IMaasa1 traditional fermented milk in Kenya. Int. J. Food Mcrobiol., 94: 269-278.

Medina, R., M. Katz, S. Gonzalez and G. Oliver, (2001). Characterization of the lactic acid bacteria in ewek milk and cheese from northwest Argentina. J. Food. Prot. 64: 759-563.

Rosenthal, I., (1991). Milk and Dairy Products: Properties and Processmg. NCH Publisher, New York, ISBN-13: 9783527279890, Pages: 218.

Saleh, F.A. (2010). Antibacterial activity of Sameel milk "Saudi traditional fermented milk. Proceedings of the 11th Egyptian Conference of Dairy Science and Technology, -November 1-3, 2010, Cairo, Egypt, pp. 407-422.

Shuangquan, B., B. Yu and T. Miyamoto (2006). Mcroflora in traditional starter cultures for fermented milk, hurunge, from Inner Mangolia, China. Anim. SCI, J. 77: 235-241.

Sulieman, A.M.E., AA. Ilayan and A.E. Elfaki (2006). Chemical and microbiological quality of Garris, Sudanese fermented camel's milk product. Int. J. Food Sci. Technol., 41: 321-328.

Saleh, F.A. (2010). Antibacterial activity of Sameel milk "Saudi traditional fermented milk. Proceedings of the 11th Egyptian Conference of Dairy Science and Technology, -November 1-3, 2010, Cairo, Egypt, pp. 407-422.

Shuangquan, B. , B. Yu and T. Miyamoto (2006). Mcroflora in traditional starter cultures for fermented milk, hurunge, from Inner Mangolia, China. Anim. SCI, J. 77: 235-241.

Sulieman, A.M.E., AA. Ilayan and A.E. Elfaki (2006). Chemical and microbiological quality of Garris, Sudanese fermented camel's milk

product. Int. J. Food Sci. Technol., 41: 321-328.

Teuber, M. and A. Geize (1981). The Family Streptococcaceae (Nonmedical: Aspects). In: The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria, Volume 2, Starr, M.P., H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (Eds.). Springer, New York, pp: 1614-1630.

Saleh, F.A. (2010). Antibacterial activity of Sameel milk "Saudi traditional fermented milk . Proceedings of the 11th Egyptian Conference of Dairy Science and Technology, -November 1-3, 2010, Cairo, Egypt, pp.407-422.

Shuangquan, B., B. Yu and T. Miyamoto (2006). Mcroflora in traditional starter cultures for fermented milk, hurunge, from Inner Mangolia, China. Anim. SCI, J. 77: 235-241.

Sulieman, A.M.E., AA. Ilayan and A. E. Elfaki (2006). Chemical and microbiological quality of Garris, Sudanese fermented camel's milk product. Int. J. Food Sci. Technol., 41: 321-328.

Teuber, M. and A. Geize (1981). The Family Streptococcaceae (Nonmedical: Aspects). In: The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria, Volume 2, Starr, M.P., H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (Eds.). Springer, New York, pp: 1614-1630.