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HEAT STABILITY OF ANTIBIOTICS RESIDUES IN MILK

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ABSTRACT

The main aim of study was to assess heat stability of antibiotics residues in the milk. Milk samples were divided into five equal parts and encoded with A, B, C, D and E. Samples encoded A were spiked with penicillin G, B with oxytetracyline, C with gentamycin and D with sulfonamide each of at 500, 750 and 1000 ppm concentration. Samples encoded E were kept as control and no antibiotic was spiked. All the spiked and control samples were sub-divided into four parts. Spiked and control samples under part one, two and three were observed for the effect of heat treatments (60 °C for 15 sec, 65 °C for 30 min and 110 °C for 10 min) on the stability of antibiotics, while samples under part four were kept as non-heated for comparison purpose. Results showed mean recovery of sulfonamide (81.73 ± 1.44) significantly higher than penicillin G (80.63 ± 0.92%), gentamycin (76.32 ± 1.29) and oxytetracycline (73.94 ± 1.56%) from spiked milk samples (65 °C) compared of non-heated antibiotics spiked milk samples. The mean recovery for sulfonamide residues (64.48 ± 1.41%) was remarkably ($P < 0.05$) higher compared to residues of penicillin G (62.93 ± 1.24%), oxytetracycline (58.90 ± 1.13%) and gentamycin (40.00 ± 1.26%) from the heated (110 °C) antibiotics spiked milk compared to non-heated spiked milk samples with similar drugs (13.80 ± 1.28, 26.27 ± 0.79, 18.43 ± 1.04, 15.60 ± 1.39 mm). On the basis of findings of this study it could be concluded that oxytetracyline, gentamycin and penicillin G residues in milk are significantly ($P < 0.05$) reduced with the pasteurization (at 65 °C) and sterilization (at 110°C). Further, sulfonamide is more thermo stable and oxytetracycline is the less at 110 °C. The prevention of antibiotics residues in milk and heat applications have strong correlation with each other.

1.Introduction

Milk is a very nutritious food with higher level of carbohydrates, protein, fats, vitamins and minerals. It can be associated with health risks to consumers due to presence of zoonotic pathogens and antimicrobial drug residues. Consumers are much conscious about their safe food supply, which should be free from contamination, herbicides, pesticides, drugs and antibiotics, though otherwise may create severe health hazards, allergic reactions, carcinogenicity and bacterial resistance (Abbas *et al*. 2013). The misuse of antibiotics in animals lead to the development of resistant bacterial strains, and their transmission to human beings result significantly reduced

efficacy of antibiotics. The antibiotics residues in milk are of great concern for dairy farmers, milk processors, consumers and regulatory agencies. The inactivation of these antibiotics residues is utmost important due to possible development of antibiotics resistance in humans (Berends *et al*. 2001).

Antibiotics are commonly used in domestic animals for the prevention and treatment of diseases. They are also used as growth promoters for enhancing the production of food producing animals. Excessive or improper use of antibiotics is of great concern due to appearance of residues in the milk (Shetandi and Sternesjo 2004). Antimicrobial residues in milk may pose potential health threats to the consumers in form of antibiotics resistance and allergies. They also influence the dairy industry by impairing the bacteriological processes used for manufacturing of dairy products (Conesa *et al*. 2008). The presence of antimicrobial substances in milk even in low concentrations is one of the main concerns of milk industry, as it poses risk of toxicity to public health, and can seriously influence the technological properties of milk and dairy products. For example, in the production of fermented milk products such compounds inhibit the growth of starter cultures (Jones 2008). Concentration of 1 ppb delays the starter activity and decreases the acid and flavors the production during yoghurt or butter making, and also causes improper ripening of cheese. In past studies, heat treatment has been applied to reduce the level of antibiotics residues in milk (Khopaibool 2015). However, other study showed that antibiotics residues may not be totally destroyed under normal cooking procedures (Ghidini *et al*. 2002). The control of residues of veterinary drugs in food producing animals and animal products has been a cornerstone of the present agricultural and food policies for providing assurance to consumers about the safety and wholesomeness of their food (Moats 2007). In order to be safeguard human health, many countries have set up maximum residue limits (MRLs) of some antibiotics in milk. Milk samples exceeding prescribed MRL for penicillin G 4 μg/l, sulfonamide MRL 100 μg/l and oxytetracyline 100 μg/l, must be excluded from human consumption (Brady 2016). Failure to adhere these recommended periods has been reported to be the primary cause of violate levels of veterinary drugs in food (KuKanich *et al*. 2005).

Present study was therefore planned in order to observe the extent of antibiotics residues in the market milk and assess influence of various heat treatments on stability of antibiotics residues in milk.

2.Materials and methods

The present study was conducted during the year 2018, whereby antibiotics free milk samples were collected from the local markets of Sindh province and brought to the laboratory of department of Animal Products Technology for analysis purpose.

2.1.Preparation of milk samples

Milk samples were divided into five equal parts and encoded as A, B, C, D and E. Samples encoded A were spiked with penicillin G (Amino-Vet, manufac. ICI, Pakistan), B with oxytetracyline (Oxytetracycline, manufac. ICI, Pakistan), C with gentamycin (Refobacin, manufac. ICI, Pakistan), D with sulfonamide (Trisolizin, manufac. ICI, Pakistan) each of at concentration of 500, 750 and 1000 ppm (AOAC 2000)[11], however samples encoded E were kept as control and no antibiotics were spiked. All the spiked and control samples were again sub-divided into four parts. Spiked and control samples under part one, two and three were observed for the effect of heat treatment (thermization; 60 °C for 15 seconds, pasteurization; 65 °C for 30 min and sterilization; 110 °C for 10 min) on the stability of antibiotic residues, while under part four samples were kept as non-heated for comparison purpose. Turbidity test was used in order estimate microbial growth.

2.2.Preparation of media and isolation of *Bacillus subtilis*

Nutrient agar was used against *Bacillus subtilis* [(Ehrenberg) Cohn, ATCC® 6051™]. Petri dishes containing nutrient agar were inoculated with test *Bacillus subtilis*. Milk samples were streaked on nutrient agar and petri dishes were incubated at 35°C under aerobic condition for 24 hours. Isolated colonies of *Bacillus subtilis* were picked-up and kept on clean glass slides. Gram staining was used for morphological characteristics of the isolates. The plates with purified colonies were stored in cryotube for further use. Moreover, purified colonies were picked from nutrient agar and inoculated in nutrient broth (HiMedia M002-100G Nutrient Broth, USA). Broth was incubated (36 ± 1 °C) for 24 hours. Before screening of test samples, this medium (0.1 ml) was mixed with soft agar (3 ml) and spread over nutrient agar (DeJonghe *et al*. 2010).

2.3.Screening of milk samples for the presence of antibiotics residues

A total of 10 samples were prepared and processed for assessing the effect of different heat treatments on stability of penicillin G, oxytetracyline, gentamycin and sulfonamide residues in milk. The blank disc of filter papers (Whatman 1, 12 mm, CAT No. 1002-125, Bharat Instruments & Chemicals Company, India) were completely dipped into each antibiotics spiked and control milk samples using forceps, and placed on the surface of agar medium containing the sensitive test organism (*Bacillus subtilis*). The plates were incubated (36 ± 1 °C) for 24 hours. The positive results (the presence of antibiotic residues) were manifested by formation of transparent zones around the disc and compared with control group. The zone size around each positive sample was measured with help of Vernier caliper. Since no any zone was appeared in control samples (no addition of antibiotics), the data on the zone size of heated and non-heated spiked milk samples were gathered for comparison purpose.

2.4.Statistical analysis

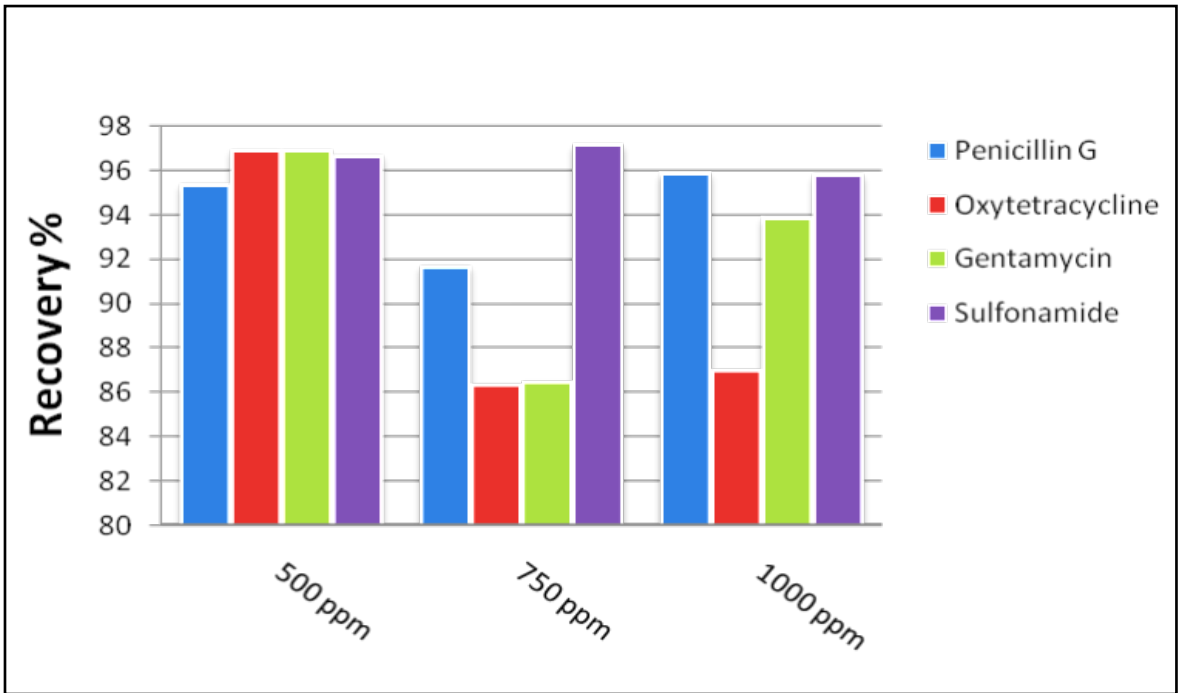
A computerized statistical package i.e. Student Edition of Statistix (SXW), Version 8.1 (Copyright 2005, Analytical Software, USA) was applied to assess the data. Statistical procedure of completely randomized analysis of variance (ANOVA) under linear models was used to observe the significant variations among the variables, and in case of the significant differences found among the means, the least significant difference (LSD) test was applied (Gomez and Gomez 1984). Difference was considered significant at ($P < 0.05$).

3.Results

In present study, the stability of antibiotics residues in milk on different heat treatments was assessed. The results obtained are given in the subsequent sections.

3.1.Penicillin G

It was observed that the zones size of penicillin G spiked (500 - 1000 ppm) milk samples were significantly ($P < 0.05$) reduced when heated at 60, 65 and 110 °C compared to non-heated spiked (penicillin G) milk samples. The average recovery percentages of penicillin G spiked milk samples with concentration of 500, 750 and 1000 ppm heated at 60 °C were 95.36, 91.67 and 95.86% (reduction level) compared to non-heated (23.60, 26.40 and 28.80 mm) spiked milk samples with similar concentrations (Figure 1).



LSD (0.05) = 3.28, S.E = 1.61 (750 ppm)
LSD (0.05) = 3.53, S.E = 1.74 (1000 ppm)
Data are the average of ten samples and each in duplicate

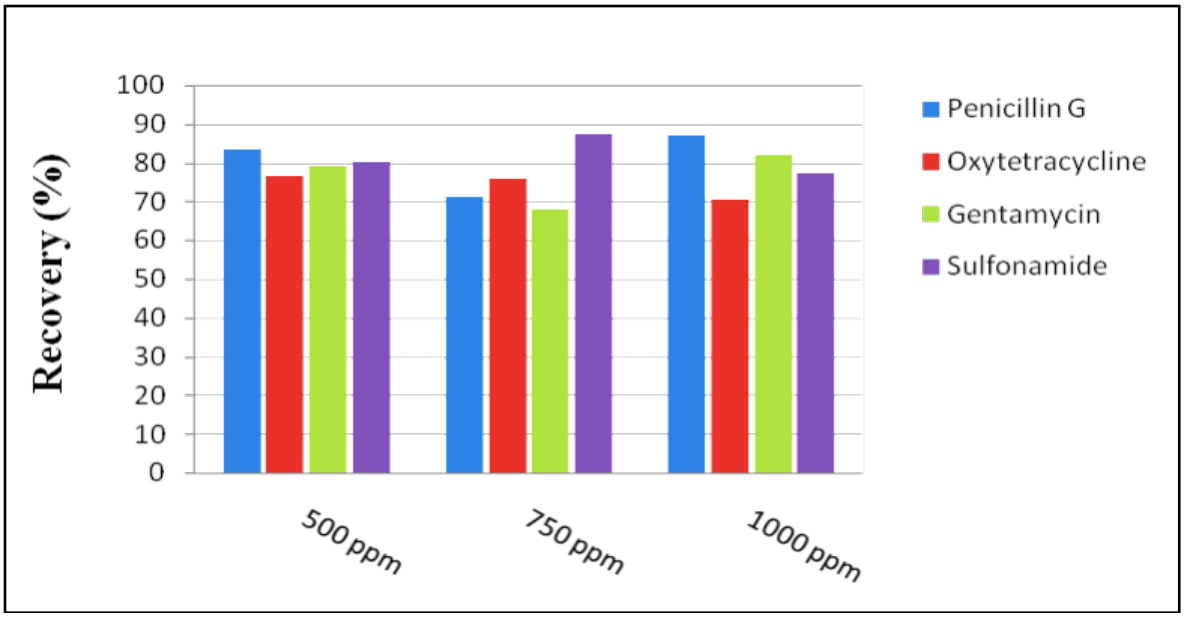
Figure 1. Recovery (%) of antibiotics (penicillin G, oxytetracyline, gentamycin and sulfonamide) residues from spiked milk (500, 750, 1000 ppm) heated at 60 °C for few seconds.

The recovery percentages in zones size of penicillin G spiked (500, 750 and 1000 ppm) milk samples heated at 65 °C were found 83.53, 71.22 and 87.16%, respectively with overall reduction of 16.47, 28.78 and 12.84%, respectively as shown in Table 1. However, at 110 °C recovery percentages in zone size were recorded 73.31, 64.06 and 51.41%, respectively (Figure 2) with average reduction of 26.69, 35.94 and 48.59% (Table 1). Mean reduction percentage in zone size of penicillin G spiked (500 - 1000 ppm) samples was highest (37.07%) at 110 °C followed by at 65 °C (19.36%) and at 60 °C (5.70%) compared to zone size of non-heated penicillin G spiked milk samples (500 - 1000 ppm) as shown in Table 2.

3.2.Oxytetracycline

In the present study, average zones size of oxytetracycline spiked milk samples (500, 750 and 1000 ppm) were detected as 12.60, 16.00 and 18.20 mm, respectively. The zones size significantly ($P < 0.05$) reduced in oxytetracycline spiked milk samples heated at 60, 65 and 110 °C. The average recovery levels in oxytetracycline spiked milk (with concentration of 500, 750 and 1000 ppm) were 96.91, 86.31 and 86.96% with average reduction level of 3.09, 13.69 and 13.04%, respectively at 60 °C (Figure 1 and Table 1). Antibiotics residues in oxyteracycline spiked milk heated at 65 °C were observed as 76.44, 75.84 and 70.36% with reduction in zones size of 23.56, 24.16 and 29.64%, respectively (Figure 2). However, at 110 °C residues reduced from 58.86, 60.68 and 57.77% to 41.14, 39.32 and 42.23%, respectively compared to non-heated oxytetracycline spiked milk samples (12.60, 16.00 and 18.20 mm) with similar concentration level (Figure 3 and Table 1). The average zone size of oxytetracycline spiked (500 - 1000 ppm) milk samples heated at 110 °C was reduced up to 41.10%. Sample heated at 65 °C and 60 °C showed reduction in zones size up to 26.06 and

10.18%, respectively compared to average zone size of non-heated oxytetracycline spiked milk samples with similar concentration (15.60 mm) as tabulated in Table 2. Present results suggested that heating of milk up to 110 °C does not completely eliminate oxytetracycline residues level.

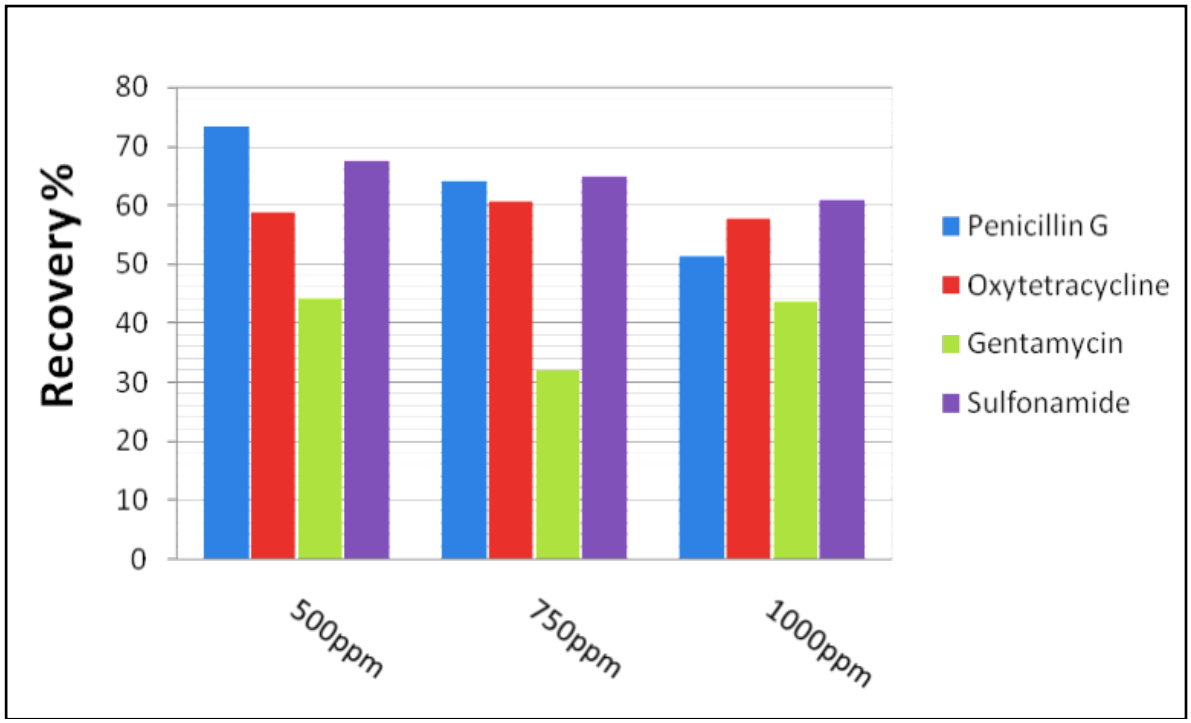


LSD (0.05) = 4.91, S.E = 2.42 (500 ppm)
LSD (0.05) = 3.72, S.E = 1.83 (750 ppm)
LSD (0.05) = 2.92, S.E = 1.44 (1000 ppm)
Data are the average of ten samples and each in duplicate

Figure 2. Recovery (%) of antibiotics (penicillin G, oxytetracyline, gentamycin and sulfonamide) residues from spiked milk (500, 750, 1000 ppm) heated at 65 °C for 30 min.

3.3.Gentamycin

Average zones size of non-heated gentamycin spiked milk samples (500, 750 and 1000 ppm) in the present study appeared as 15.80, 18.40 and 21.10 mm, respectively (Table 1). The zones size were remarkably ($P < 0.05$) reduced in gentamycin spiked milk samples heated at 60, 65 and 110 °C. The average recovery percentages of antibiotics residues from gentamycin spiked milk (500, 750 and 1000 ppm) at 60 °C appeared 96.87, 86.43 and 93.87% with decreased zone size of 3.13, 13.57 and 6.13% (Figure 1). The recovery percentages of residues from gentamycin spiked milk samples heated at 65 °C revealed as 79.20, 67.98 and 82.07% with zone size reduction 20.80, 32.02 and 17.93% (Figure 2 and Table 1). Whereas spiked milk heated at 110 °C (Figure 3) revealed recovery of antibiotics residues 44.33, 32.08 and 43.61% with zones size reduction up to 55.77, 67.92 and 56.39%, respectively compared to non-heated gentamycin spiked milk samples (500, 750 and 1000 ppm). The average reduction in zones size of gentamycin spiked milk samples (500 - 1000 ppm) heated at 110 °C was highest (60.03%) followed by heated at 65 °C (23.58%) and at 60 °C (7.61%) as shown in the Table 1.



LSD (0.05) = 3.96, S.E = 1.95 (500 ppm)
LSD (0.05) = 4.17, S.E = 2.05 (750 ppm)
LSD (0.05) = 3.01, S.E = 1.48 (1000 ppm)
Data are the average of ten samples and each in duplicate.

Figure 3. Recovery (%) of antibiotics (penicillin G, oxytetracyline, gentamycin and sulfonamide) residues from spiked milk (500, 750, 1000 ppm) heated at 110 °C for 10 min.

3.4.Sulfonamide

Sulfonamide spiked milk (500, 750 and 1000 ppm) heated at 60 °C appeared as 96.66, 97.14 and 95.77% (decreased to 3.34, 2.86 and 3.33%) of total non-heat zone size respectively (11.60, 13.40 and 16.40 mm) (Figure 1 and Table). The recovery residues percentages were 80.34, 87.47 and 77.42% and that reduced to 19.66, 12.53 and 22.58% when heated at 65 °C. At 110 °C observed residues recovery percentage 67.44, 65.00 and 61.00% reduced to 32.56, 35.00 and 39.00%, respectively (Figure 2 and Figure 3). The mean reduction level in zones size of sulfonamide spiked milk (500 - 1000 ppm) samples was higher at 110 °C (32.52%) and lower at 60 °C (3.48%) with intermediate level of 18.25% at 65 °C compared to mean zones size of non-heated sulfonamide spiked milk samples (13.80 mm) as summarized in Table 2. Findings further indicate that thermization process (60 °C for 15 seconds) has no significant ($P > 0.05$) effect on the reduction of residues in sulfonamide contaminated milk. While sterilization process (110 °C for 10 min) reduces up to 50% level of residues in sulfonamide spiked milk. Severe heating is required for complete reduction of sulfonamide residues from milk.

Table 1. Effect of various heat treatments on antibiotics (penicillin G, oxytetracycline, gentamycin and sulfonamide) spiked milk samples (500, 750 and 1000 ppm)

Concentration	Zone size of penicillin G (mm) 500 ppm				Zone size oxytetracycline (mm) 500 ppm			
	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)
Maximum	24.00	23.00	20.00	18.00	14.00	13.00	11.00	8.00
Minimum	23.00	22.00	19.00	16.00	12.00	11.00	9.00	7.00
Mean	23.600	22.500	19.700	17.300	12.60	12.200	9.600	7.400
SE±	0.163	0.163	0.152	0.213	0.221	0.2000	0.221	0.163
Concentration	**Zone size of penicillin G (mm) 750 ppm**				**Zone size oxytetracycline (mm) 750 ppm**			
	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)
Maximum	27.00	25.00	20.00	19.00	17.00	15.00	13.00	10.00
Minimum	26.00	23.00	18.00	15.00	15.00	13.00	11.00	9.00
Mean	26.400	24.200	18.800	16.900	16.00	13.800	12.00	9.700
SE±	0.163	0.249	0.249	0.504	0.210	0.2000	0.210	0.152
Concentration	**Zone size of penicillin G (mm) 1000 ppm**				**Zone size oxytetracycline (mm) 1000 ppm**			
	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)
Maximum	29.00	28.00	26.00	16.00	19.00	17.00	14.00	10.00
Minimum	28.00	27.00	24.00	14.00	17.00	15.00	12.00	11.00
Mean	28.800	27.600	25.100	14.800	18.200	15.800	12.800	10.400
SE±	0.133	0.163	0.233	0.200	0.2000	0.2000	0.2000	0.163
Concentration	**Zone size of gentamycin (mm) 500 ppm**				**Zone size sulfonamide (mm) 500 ppm**			
	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)
Maximum	16.00	16.00	13.00	8.00	13.00	12.00	10.00	8.00
Minimum	15.00	15.00	12.00	6.00	11.00	10.00	9.00	7.00
Mean	15.800	15.300	12.500	7.00	11.600	11.300	9.300	7.800
SE±	0.133	0.152	0.166	0.210	0.221	0.213	0.152	0.133
Concentration	**Zone size of gentamycin (mm) 750 ppm**				**Zone size sulfonamide (mm) 750 ppm**			
	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)
Maximum	19.00	17.00	13.00	7.00	13.00	12.00	10.00	8.00
Minimum	18.00	15.00	12.00	5.00	11.00	10.00	9.00	7.00
Mean	18.400	15.900	12.500	5.900	11.600	11.300	9.300	7.800
SE±	0.163	0.179	0.166	0.233	0.221	0.213	0.152	0.133
Concentration	**Zone size of gentamycin (mm) 1000 ppm**				**Zone size sulfonamide (mm) 1000 ppm**			
	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)	Non heated	Heated (60 °C)	Heated (65 °C)	Heated (110 °C)
Maximum	22.00	21.00	18.00	10.00	17.00	17.00	14.00	11.00
Minimum	20.00	19.00	17.00	8.00	16.00	15.00	12.00	9.00
Mean	21.100	19.800	17.300	9.200	16.400	15.800	12.700	10.00
SE±	0.179	0.249	0.152	0.249	0.163	0.200	0.213	0.210

Table 2. Effect of various heat treatments on antibiotics (Penicillin G, Oxytetracycline, Gentamycin and Sulfonamide) spiked milk samples (500 - 1000 ppm)

Heat treatment at 60 °C					
Antibiotics	No heat zone size (mm)	zone size (mm)	Recovery (%)	Reduction (%)	Standard error
Penicillin G	26.27	24.76	94.29	5.76	0.79
Oxytetracycline	15.60	13.93	90.0	10.18	1.39

			6		
Gentamycin	18.43	17.00	92.39	7.61	1.04
Sulfonamide	13.80	13.34	96.52	3.48	1.28
Heat treatment at 65 °C					
Antibiotics	No heat zone size (mm)	zone size (mm)	Recovery (%)	Reduction (%)	Standard error
Penicillin G	26.27	21.20	80.63	19.36	0.92
Oxytetracycline	15.60	11.47	73.94	26.06	1.55
Gentamycin	18.43	14.10	76.32	23.58	1.29
Sulfonamide	13.80	11.23	81.73	18.25	1.44
Heat treatment at 110 °C					
Antibiotics	No heat zone size (mm)	zone size (mm)	Recovery (%)	Reduction (%)	Standard error
Penicillin G	26.27	16.33	62.93	37.07	1.24
Oxytetracycline	15.60	9.17	58.90	41.10	1.13
Gentamycin	18.43	7.37	40.00	60.00	1.26
Sulfonamide	13.80	8.83	64.48	32.52	1.41

3.5.Discussions

Current study was carried out for observing the stability of antibiotics residues in the milk with respect to different heat treatments. It was observed that average recovery percentage of penicillin G spiked milk samples (500, 750 and 1000 ppm) heated at 60 °C was 95.36, 91.67 and 95.86% compared to non-heated spiked milk samples. Recovery percentages of zones size 65 °C were 83.53, 71.22 and 87.16%. However, at 110 °C zones size were recorded 73.31, 64.06 and 51.41%. Mean reduction percentage in zones size of penicillin G spiked (500 - 1000 ppm) samples was highest at 110 °C followed by at 65 °C and at 60 °C compared to non-heated spiked milk samples. Our results are not in consistent with the findings of (Loksuwan 2002) who reported non-significant ($P > 0.05$) reduction in penicillin G spiked milk (500 - 1000 ppm) heated at 65 °C for 30 min. The variation in the results may be due to change in the efficacy of antibiotics with respect to producing company as well experimental site. Change in the results may also because of heat treatment methods used during the research. Our results are supported by another research, where effects of heat treatments on stability of β-lactams in milk were studied. Their results indicated that in sterilization, the heat treatment of 120°C for 20 min leads to high degradation of β-lactam antibiotics including penicillin G. The degradation rates of penicillin remains between 47.6% and 84% (Roca *et al*. 2011). Results obtained by Zorraquino *et al*. (2008) are also supportive to present study. They indicated that pasteurization at 63 °C for 30 min shows low antimicrobial activity loss for penicillin (between 7% and 11%) compared to cephalosporins (between 6% and 18%). Our

results show significant relevance with (Konecˇny 2007), who observed low-heat inactivation percentage (10%) in penicillin G–fortified milk samples heated at 83 °C for 10 min. This percentage increased to 30 and 32% when milk was treated at 70 °C for 30 min and 100 °C for 30 min, respectively. Jacquet and Auxepaules (2012) carried out a low temperature–long-time pasteurization (63 °C for 30 min). They obtained low inactivation percentages for ampicillin (1.7%) and penicillin G (2.6%); values supportive to those that appear in this study. Tropilo (2015), on the other hand, obtained an inactivation percentage of 15.2% in aqueous penicillin solutions at pH 7.0 when heated at 80 °C for 15 min. However, when these solutions were heated to 121 °C for 15 min, the percentage increased greatly to 81.5%. If we compare them to what is obtained using milk samples, then this increase in the inactivation percentage could be attributed to a possible protective effect of the milk's fat content on the antimicrobial molecules. The work of Broggler *et al*. (2015) indicated that if a moderate dose of penicillin (50,000 units) is administered, a significant amount (0.14 units/ ml) is detectable in milk from the treated animal 72 hours later. Further, penicillin has been reported in one of the first reported surveys of antibiotics in market milk which was conducted in New York State in 1951 (Kosikowsky *et al*. 2013). A total of 1,794 samples of fresh, blended and pasteurized whole milk were obtained from dairy plants or route wagons in 36 counties. Six percent of the samples tested contained antibiotics and they were present in the range of 0.05 - 0.1 unit per ml of milk. Penicillin was found most frequently appeared antibiotic in the milk during the spring of the year. The first survey was made on both raw and pasteurized milk obtained from seven of the 16 Food and Drug Administration districts and showed that 3.2% of 94 samples contained penicillin and 1.07% contained bacitracin. These all reports strongly support to our results (Welch 2005; Welch *et al*. 2010).

In present study, the average recovery levels in oxytetracycline spiked milk (500, 750 and 1000 ppm) were observed 96.91, 86.31 and 86.96% with average reduction levels of 3.09, 13.69 and 13.04% at 60 °C. Antibiotics residues at 65 °C were observed 76.44, 75.84 and 70.36%, however at 110 °C residues reduced. The average zones size of oxytetracycline spiked (500 - 1000 ppm) milk samples heated at 110 °C showed reduction up to 41.10%, while at 65 °C and 60 °C reduction was up to 26.06 and 10.18%. Loksuwan (2002) studied the effects of low-temperature long-time (LTLT) pasteurization (63 °C/30 min) on oxytetracycline residues in raw milk. The oxytetracycline residues were in samples with concentration of 100 $\mu g \cdot l^{-1}$ inactivated to such an extent that they could not be detected. The starting oxytetracycline concentrations were found to be dropped by 86.7%. Hsieh *et al*. (2011) studied the effects of the heat treatments on tetracycline thermostability, using double-distilled water as a matrix. They used two different heating temperatures (100 °C, 121 °C) with the same time of exposure (15 min). Their findings showed that higher temperatures (121 °C/15 min) cause tetracycline degradation of up to 99%. At 100 °C the degradation was less extensive, amounting to as little as 54.4%. The results of this study clearly show that the degree of oxytetracycline degradation is temperature dependent and these same results have been observed in our study. In support our study, Hassani *et al*. (2008) set out to determine the thermostability of oxytetracycline in McIlvaine buffer with varying pH value (pH 7.0, 5.5 and 4.0) and ultra-high temperatures ranging from 110 to 140 °C. The results of the study showed that sterilization (118 °C per 30 min and 121 °C per 20 min) reduces the concentration of oxytetracycline to negligible amounts (less than 0.01%). The ultra-high temperature treatment on the other hand, reduces oxytetracycline concentrations by more than 40%. At 135 °C per 15 sec the ultra-high temperature treatment inactivate oxytetracycline residues by 44%. While the sterilization process degrades oxytetracycline in

milk by more than 98%. Few others reported that pasteurization of milk which contained antibiotics did not inactivate chlortetracycline, chloramphenicol, streptomycin and oxytetracycline (Overby 2015). These results are somewhat change from our study. Difference may be related to the variation in heat treatment methods. Shahani (2011) reported a reduction of 9.3, 14.2, 18.4, 22.5 and 25.4 percent in the potency of chlortetracycline in milk which were pasteurized at 143 °F for 10, 20, 30, 40 and 50 minutes, respectively. The loss in potency was 13.5, 20.1, 27.1, 32.5 and 38.2 percent when milk were pasteurized at 160 °F for the same time periods. These results strongly agree with our reports. More recently it has been found that pasteurization at 143 °F for 30 minutes result 30 percent loss of oxytetracycline activity when 0.84 to 1.0 ug of the antibiotics is present per ml of milk. An increase in pasteurization temperature to 160 °F results in a 4 < percent loss in activity after 30 minutes of heating. Oxytetracycline in milk is completely inactivated by heating to 160 °F for 190 minutes, 175 °F for 92 minutes and 185 °F for 60 minutes (Shahani 2009). These results strongly support to our findings regarding oxytetracycline.

Further, the average recovery percentages of antibiotics residues from gentamycin spiked milk (500, 750 and 1000 ppm) at 60 °C appeared 96.87, 86.43 and 93.87% with decrease zones size of 3.13, 13.57 and 6.13%. The recovery percentages of residues at 65 °C were 79.20, 67.98 and 82.07%, whereas, at 110 °C were 44.33, 32.08 and 43.61%. The average reduction in zones size of gentamycin spiked milk samples (500 - 1000 ppm) heated at 110 °C was highest followed by heated at 65 °C and 60 °C. Our findings are in consistent with the study of (Omar and Eltinay 2008). They reported same reduction pattern of gentamycin as investigated in current research. Few other authors reported that heat treatment at 60 °C for 30 min does not inactivate the residues of gentamycin, while classic sterilization (120 °C for 20 min) shows high heat inactivation (> 95%) for the residues of gentamycin.

Sulfonamide spiked milk (500, 750 and 1000 ppm) heated at 60 °C appeared as 96.66, 97.14 and 95.77%. The recovery residues percentages were 80.34, 87.47 and 77.42% at 65 °C, while 67.44, 65.00 and 61.00% at 110 °C. Present findings indicated that thermization process has no significant effect on the reduction of residues in sulfonamide contaminated milk. While sterilization process (110 °C for 10 min) reduces up to 50% level of residues in sulfonamide spiked milk. Our results are supported by (Yassin *et al*. 2015) who stated that N^4 aromatic group of sulfonamide reacts with reducing sugar to form a sugar sulfonamide complex which is more stable to heating at 100 °C. Results are also supported by (Malik 2014), who reported that heat treatment up to 100 °C causes lower reduction of sulfonamide residues. It may be due to binding of sulfamethazine to protein and reducing sugars.

4.Conclusions

On the basis of present findings it could be concluded that thermization (60 °C) poorly reduces antibiotics residues in milk. However penicillin G, oxytetracyline and gentamycin residues in milk are considerably reduced with the pasteurization (65 °C) and sterilization (110 °C). Thermostability of sulfonamide residues remains higher compared to penicillin G, oxytetracyline and gentamycin residues. It was further concluded that complete elimination of antibiotics residues from milk by thermization, pasteurization and sterilization is not possible, however residues levels and their efficacy can be reduced. It is suggested to boil the milk at higher temperature in order to reduce the concentration as well as efficacy of antibiotics residues in milk, though otherwise may develop antimicrobial resistance in the consumers.

5.References

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