Virulence and multi-resistance of gram-negative bacilli strains isolated from some artisanal fermented dairy products sold in secondary schools in Benin

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This work aims at assessing toxin production capability and antibiotic resistance profiles of thermo-tolerant Gram-negative bacilli strains isolated from three types of fermented dairy products (yoghurt, dèguè millet and dèguè couscous). Samples collected in Abomey-Calavi and Cotonou were analyzed for microbial, biochemical and molecular parameters. Samples were contaminated with thermo-tolerant Gram-negative bacilli strains at 13.88%. The high contamination rate was recorded with the samples of dèguè couscous and the lowest contaminated samples were dèguè millet. Morning samples were more contaminated. Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli and Enterobacter cloacae were the most identified bacteria. The most isolated species in the dry season was E. coli. In the rainy season, K. pneumoniae and E. cloacae were the most isolated species. K. pneumoniae was the most formative of biofilm (38.9%). About 12% of the isolated strains were extended-spectrum beta-lactamases (ESBL) producers. The higher resistance rate was observed with amoxicillin and doxycyclin (92.3%). Imipenem was the most efficient molecule on the isolated strains with 00% of resistance. The blaTEM gene was detected in 51.43% of the isolated strains followed by blaSHV (37.14%), blaCTX-M (8.57%) and blaOXA-1 (2.86%). It is necessary to train sales people on good hygiene practices for food during their production and their distribution.

Key words: Fermented milk products, thermo-tolerant gram-negative bacilli, toxins, antibiotics resistance, Benin.

INTRODUCTION

Milk is a complex ecosystem for various microorganisms including bacteria. The widely consumed milk products are cheese, yoghurt and curdle milk (Pal and Awel, 2014). Dairy products undergo lactic fermentation to
ensure food safety through acidification and production of bacteriocins that antagonize the growth of pathogenic bacteria, and improve the final quality of dairy products by producing aromatic compounds. There is an increase demand of natural food without any artificial additive and pathogenic microorganisms. However, several germs such as yeasts and bacteria are responsible for altering the marketable and hygienic quality of these dairy products (Abdel-Aziz et al., 2016).

Food borne pathogens cause outbreak of food borne diseases through pathogenic microorganisms or their toxins (Smith and Fratamico, 2018). Thus, in developing countries, foodborne infections remain a major public health problem since they can affect a large part of population (Djogbe et al., 2019). Milk and their derived products are important sources of food borne pathogen such as bacteria (Oliver et al., 2005; Zagare et al., 2012). In Benin, traditional techniques for improving preservation and production of yoghurt and dégou are expensive and less accessible to processors (Sessou et al., 2013; Tohoyessou et al., 2020).

Milk and its derivate products contamination by pathogenic bacteria is largely due to processing, handling, and unhygienic conditions. Garas et al. (2017) describe the presence of microorganisms that can cause food poisoning are viruses and bacteria. These include thermo-tolerant Gram-negative bacilli such as Clostridium botulinum, Clostridium perfringens, Campylobacter, Salmonella and Escherichia coli (Mon et al., 2015). Nowadays, food poisoning and safety is very important subject all over the world.

The Gram-negative bacilli in particular Enterobacteriaceae are becoming increasingly resistant to antibiotics particularly to carbapenems (Pfeifer et al., 2010; Nordmann et al., 2011). Carbapenems remain the beta-lactam with the broadest spectrum of activity. Their excellent antibacterial activity is linked to their rapid transmembrane penetration through the external wall of Gram-negative bacilli. In addition, the have stability against most natural or acquired beta-lactamases, including chromosomal and plasmid cephalosporinases and extended spectrum beta-lactamases (Nordmann and Carrer, 2010).

Considering the numerous cases of food poisoning induced by the multi-resistant bacteria, it is necessary to investigate the resistance ability of potentially pathogenic microorganism founded in foods such as fermented milk products. Thus, the aim of this work was to identify and characterize thermo-tolerant Gram-negative bacilli isolated from three artisanal fermented dairy products (yoghurt, dégou millet and dégou couscous) collected from the schools of Abomey-Calavi and Cotonou (Benin).

MATERIALS AND METHODS

Sampling and samples collection

The three dairy products (yoghurt, dégou millet and dégou couscous) samples were randomly collected from 15 schools of Abomey-Calavi and Cotonou (Figure 1). Those samples were preferentially taken from inside schools’ vendors. For each products, two samples were taken two time daily (morning and evening) and repeated twice in a week with. Thus, 180 samples (60 yoghurt, 60-dégou couscous and 60-dégou millet) were collected during this study. Collected samples were carried, in ice (about 4°C), to the laboratory for further analysis.

Microbiological analyses

Each collected sample (10 g) was aseptically homogenized into sterile tryptone salt water (90 ml). From this solution, a serial decimal dilution was made to obtain dilution 10⁻¹, 10⁻², 10⁻³, up to 10⁻⁷. For the detection and enumeration of total and fecal coliforms, dilutions 10⁻¹ to 10⁻³ were used.

Total and fecal coliforms

Total and fecal coliforms were enumerated on Violet Red Bile Glucose Agar (VRBA, OXOID CM0485) medium following a previously describe method (Miliari et al., 2011). Dilutions from 10⁻¹ to 10⁻³ were homogenized with 15 ml of VRBA medium and after solidify, a second stratum of about 5 ml was poured. The incubation was performed at 30°C for 24 h (total coliforms) and at 44°C for 24 h (fecal coliforms).

Thermo-tolerant Gram-negative bacilli

Tryptone Bile X Glucuronide agar and Eosin Methyl Blue Agar (OXOID, CM0089) were used for the isolation of thermo-tolerant Gram-negative bacilli. The identification of such bacilli was completed with indole test and the Api20E gallery (Riegel et al., 2006).

MALDI-TOF mass spectrometry

After isolation, the MALDI-TOF mass spectrometry was used to confirm the microbial identification. Thus, the MALDI-TOF target plate (Bruker Daltonics) was used to receive bacterial samples. The deposited samples were covered with 1.5 µl of the matrix solution (Sigma, Lyon, France) completed with acetonitrile 50% (500 µl), trifluoroacetic acid 10% (250 µl) and water (250 µl). The mixture was then sonicated for 10 min, centrifuged (13000 g, 5 min) and transferred to a clean polypropylene tube. The target plate and matrix were then dried at room temperature before MALDI-TOF/MS (BrukerDaltonics, Germany) identification (Pfeiderer et al., 2013). A positive control was used (E. coli ATCC 8739).

Biofilm formation test

The thermo-tolerant Gram-negative bacilli capacity to produce

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biofilm was determined according the method described by Christensen et al. (1985). Briefly, the 48 well microplate was used to assess, *in vitro*, qualitative biofilm production. Thus, on the microplates, 10 µl of 18 h old bacteria suspension was diluted with 150 µl of Brain Heath Infusion (BHI) and incubated for 24 h at 37°C. After incubation, wells were washed three times with sterile physiological water (0.2 ml) in order to eliminate the free bacteria. Biofilms formed by adhesion of sessile organisms to the microplate in each well are stained with crystal violet (0.1%) for 10 min. The excess dye was removed after thorough washing and the plates were left at room temperature for drying (Stepanović et al., 2000).

**Antibiotic susceptibility of strains of thermo-tolerant Gram-negative bacilli**

The susceptibility of the identified thermo-tolerant Gram-negative bacilli to 16 conventional antibiotic molecules was performed using the diffusion method (CASFM/EUCAST, 2019). The 16 antimicrobial agents (BioRad®) tested were: cefoxitin (FOX 30 µg), norfloxacin (NOR 5 µg), doxycycline (DOX 30 µg), trimethoprim sulfamethoxazole (Sxt 23.75 µg), amoxicillin-clavulamic acid (AMC 20/10 µg), amoxicillin (AMX 25 µg), ceftriaxone (CRO 30 µg), azetronam (ATM 30 µg), imipenem (IPM 10 µg), piperacillin tazobatam (PTZ 36 µg), ticarclillin (TIC 75 µg), cefepim (FEP 30 µg), ceftazidime (CZD 10 µg), ciprofloxacin (CIP 5 µg), cefotaxime (COX 5 µg) and gentamycin (GMN 10 µg).

**Penicillinase test**

The penicillinase production was detected using a tube acidimetric method test on all the isolated strains as previously described (Koneman et al., 2006). Briefly, to obtain a purplish red color, 300 µl of 1% phenol red was added to the mixture. The final reaction volume is 1 ml. Two young colonies were suspended in 500 µl of physiological water (9%NaCl). The emulsion was supplemented with 150 µl of pH 8 benzyl-penicillin (600 mg of benzyl-penicillin + 400 µl of sterile distilled water). *E. coli* ATCC 25922 was used as a control. After incubation (37°C, 1 h) orange or yellow color in the tubes indicates penicillinase production.

**Phenotypic detection of extended spectrum beta-lactamase**

To carry out this test, a bacterial suspension (10⁶ CFU/ml) was used (CASFM/EUCAST, 2019). Cefotaxim (COX 3 µg) and cefipim (FEP 30 µg) in the presence of amoxicillin + clavulanic acid (AMC) was used. AMC disc was placed in the center of Mueller-Hinton agar between the two-cephalosporins discs at a distance of about 30 mm. After incubation (37°C for 18 h), the positive result was a potentiation of the corkscrew-shaped inhibition zone between the COX and AMC discs and then between the AMC and FEP discs (CASFM/EUCAST, 2019).

**Hodge test**

To perform this test, with *E. coli* ATCC 25922 suspension (1/10 dilution of a 0.5 McFarland suspension) and ertapenem disc (10 µg). Bacteria to be tested were seeded in line at about 20 to 25 mm of distance, from the ertapenem disc to the tip of the agar. After incubation (37°C, 24 h), the shoot of *E. coli* ATCC 25922 near the ertapenem disc around the streak of the tested strain produced a depression in the agar indicating carbapenemase activity. If the growth of *E. coli* ATCC 25922 continues to be inhibited even in the...
Table 1. The PCR programs used for the amplifications.

| Genes      | Initial denaturation | Denaturation | Hybridization | Extension | Final extension | Number of cycles |
|------------|----------------------|--------------|---------------|-----------|-----------------|-----------------|
| BlaTEM     | 94°C for 5 min       | 94°C for 30 s| 52°C for 30 s | 72°C for 1 min | 72°C for 10 min | 30              |
| BlaSHV     | 94°C for 10 min      | 94°C for 40 s| 60°C for 40 s | 72°C for 1 min | 72°C for 7 min  | 30              |
| BlaCTX-M   | 94°C for 10 min      | 94°C for 40 s| 60°C for 40 s | 72°C for 1 min | 72°C for 7 min  | 35              |
| BlaOXA-1   | 94°C for 10 min      | 94°C for 40 s| 60°C for 40 s | 72°C for 1 min | 72°C for 7 min  | 30              |
| STX-1      | 94°C for 2 min       | 94°C for 1 min| 62°C for 90 s| 72°C for 1 min | 72°C for 5 min  | 30              |
| STX-2      | 94°C for 2 min       | 94°C for 1 min| 62°C for 90 s| 72°C for 1 min | 72°C for 5 min  | 30              |

Table 2. The sequences of primers used.

| Gene      | Sequences                                   | Weight (bp) | Reference        |
|-----------|---------------------------------------------|-------------|-----------------|
| BlaTEM    | 5'-TTGGGTGCACGAGTGGG TTA-3'                 | 467         | Anago et al. (2015) |
|           | 5'-TAATTGTGCGGGGAAAGCTA-3'                 |             |                  |
| BlaSHV    | 5'ATT TGT CGC TTCTTT ACT CGC-3'             | 713         | Dallenne et al. (2010) |
|           | 5' TTT ATG GCG TTACCT TTG ACC-G3'         |             |                  |
| BlaCTX-M  | 5'-ATG TGC AGYACC AGT AAR GT 3'            | 688         | Dallenne et al. (2010) |
|           | 5'-TGG GTRAAR TAR GTS ACC AGA 3'          |             |                  |
| BlaOXA-1  | 5'-ATATCTCTACTGTTGATCTCTC-3'               | 564         | Dallenne et al. (2010) |
|           | 5'-AAACCTTCAACCATC-3'                      |             |                  |
| Stx1      | 5'-GTAACTGGGAAAGTGAGTATACA-3'              | 210         | Meng et al. (1997) |
|           | 5'-GCTATTCTGAGTCAAGAAATAAC-G3'            |             |                  |
| Stx-2     | 5'-GTTTTTCTCGGTATCTATTC-3'                 | 484         | Meng et al. (1997) |
|           | 5'-GATGCATCTCGTGATTAC-G3'                  |             |                  |

Presence of the strain tested the test result is negative (Girlich et al., 2012).

Detection of genes encoding the production of β-lactamases and of toxins for E. coli

All the ESBL-producer Gram-negative bacilli were used to detect genes encoding multi-resistance (TEM, CTX-M, SHV and OXA-1). In addition, the Shiga toxins genes (Stx-1 and Stx-2) were researched for E. coli isolates. The DNA template was extracted using a boiling method. Briefly, in 500 µl of sterile water a bacteria colony was incubated at 95°C for 10 min. After, the suspension was centrifuged (1200 rpm for 5 min), and 10 µl of the supernatant was used as target DNA (Rasmussen and Morrissey, 2008). DNA extracts were used for the polymerase chain reaction (PCR) reaction. The 25 µl PCR mixture containing 12.5 µl of 2xMaster Mix (BioLabs), 1.5 µl of forward primer and 1.5 µl of reverse primer, and 4 µl of DNA was used. The PCR program used for the amplifications are in the Table 1 and the primers used are in the Table 2. After 30 min electrophoresis (150 V) on 1.5% agarose gel containing ethidium bromide, 10 µl of the PCR products were visualized with a 100 bp molecular weight ladder.

Data analysis

The MS Office Excel 2010 spreadsheet was used for data
Table 3. Bacterial load of fecal coliforms and thermo-tolerant Gram-negative bacilli (TTGNB) in samples collected in Cotonou and Abomey-Calavi.

| Sample         | fecal coliforms (UFC/ml)           | TTGNB (UFC/ml)          |
|----------------|-----------------------------------|-------------------------|
| Yoghurt        | $0.39 \times 10^5 \pm 0.05 \times 10^5$ | $7.21 \times 10^5 \pm 0.52 \times 10^5$ |
| Dégue Millet   | $11.12 \times 10^5 \pm 0.96 \times 10^5$ | $3.09 \times 10^5 \pm 0.17 \times 10^5$ |
| Dégue Couscous | $7.75 \times 10^5 \pm 0.16 \times 10^5$ | $10.81 \times 10^5 \pm 0.61 \times 10^5$ |

**Results**

Environment of the fermented milk products sale points

Some sales sites are unhealthy with the observation of rubbish and open gutters wastewater. Some vendors do not have adequate garbage bins for garbage collection. Dairy products are prepared at home and the rest of the activities carried out at the sale sites, using the water available at the site. Most vendors rarely change utensils rinse water. No vendors wear masks and gloves, so the hands of some vendors are in direct contact with money and the millet and couscous.

Microbiological quality of fermented dairy products

Enumeration of fecal coliforms and thermo-tolerant Gram-negative bacilli in sampled fermented milk products

The germ count results in the fermented milk products analyzed, in CFU/ml, were compiled in Table 3. Table 3 represent the microbial load of yoghurt, dégué millet and dégué couscous. Results indicated that the microbial loads vary according to the type of samples. For fecal coliforms, the most contaminated samples were dégué millet samples ($11.12 \times 10^5$ UFC/ml) and the least contaminated were yoghurt samples. For Gram-negative bacilli, couscous samples were the most contaminated and the least contaminated samples were those of dégué millet ($3.09 \times 10^5$ UFC/ml).

Different species of thermo-tolerant Gram-negative bacilli found in the fermented milk products

Twenty-five Gram-negative bacilli strains were isolated from the 180 samples tested. The four most represented species were strains of Klebsiella pneumoniae (38.9%), E. coli (22.2%), Enterobacter aerogenes (11.1%) and E. cloacae (11.1%). While Acinetobacter baumannii, Citrobacter sp and Klebsiella ornithinolytica strains were the least isolated (5.6%) (Figure 2).

Distribution of thermo-tolerant Gram-negative bacilli isolates according to the type of fermented milk products

Figure 3 presents the different thermo-tolerant Gram-negative bacilli of strains of obtained as a function of the processing. R 3.6.1 software was used for p-value test and for graphing. The test is considered statistically significant if $p < 0.05$. 

Figure 2. Rates of the different species of thermo-tolerant Gram-negative Bacilli identified.
fermented milk products analyzed. A total, 7 species of thermo-tolerant Gram-negative bacilli were identified. It appears that E. coli strains were the most dominant in yoghurt and dèguè couscous. In the samples of dèguè millet K. pneumoniae was the most present. It is noted a presence of six different species in dèguè couscous, five species in dèguè millet and three species in yoghurt. Enterobacter cloacae, E. coli, and K. pneumoniae were present in dèguè couscous, dèguè millet and yoghurt. The distribution of species according is not significantly different (p> 0.05).

**Figure 3.** Distribution of thermo-tolerant Gram-negative Bacilli strains according to fermented milk products.

**Figure 4.** Distribution of thermo-tolerant Gram-negative bacilli strains according to the time of collection.

**Distribution of thermo-tolerant Gram-negative bacilli according to the collection period**

Samples of dèguè couscous and dèguè millet were more contaminated in the morning than in the afternoon with respective proportions of 23.08 and 19.23% (Figure 4). The yoghurt was contaminated in the morning with a proportion of 15.38% and not in the afternoon. In general, high contamination was recorded in dry season (Figure 5). The most commonly species found in the dry season was K. pneumoniae followed by E. coli and in the rainy
season, the species most commonly found were \textit{E. coli} and \textit{K. pneumoniae}.

**Biofilm formation according to the isolated species of thermo-tolerant Gram-negative bacilli**

The biofilm production capacity by the thermo-tolerant Gram-negative bacilli strains shows that \textit{K. pneumoniae} was the most biofilm formative (38.9%) followed by \textit{E. coli} (22.2%) and \textit{Enterobacter aerogenes}, \textit{E. cloacae} (11.1%) (Figure 6). \textit{Acinetobacter baumani}, \textit{Citrobacter sp.} and \textit{Klebsiella ornithinolytica} were the lowest biofilm producers (5.6%). Meanwhile, the production of biofilm is not statistically significant different among the isolated species (p> 0.05). Gram-negative bacilli strains isolated from dèguè millet (34.62%) were the highest biofilm producer while those isolated from yoghourt were the
lowest (7.69%) (Figure 7). Biofilm production is statistically different from isolated species in function of samples types ($p=0.06$).

**Penicillinase, ESBL and carbapenemase production by the thermo-tolerant Gram-negative bacilli species**

The production of penicillinase by isolated thermo-tolerant Gram-negative bacilli strains was highly observed with *E. coli* (11.54%), followed by *Enterobacter cloacae*, *K. pneumoniae* and *Klebsiella* sp. (7.69%). *Acinetobacter baumani* and *Enterobacter aerogenes* species were not penicillinase-producers (Figure 8). Only three strains were of ESBL producers. Of the seven species isolated, only *E. coli* (7.69%) and *E. aerogenes* (3.85%) produced ESBL (Figure 9). None of the Gram-negative bacilli strains were carbapenemase-producers.
Susceptibility to antibiotics of thermo-tolerant Gram-negative bacilli strains

Most of thermo-tolerant Gram-negative bacilli were resistant to β-lactam antibiotics. The highest resistance rate (92.3%) was recorded with amoxicillin and doxycyclin followed by ticarcillin (61.5%) and amoxicillin+ clavulanic Acid (57.7%). The only sensitivity was observed with imipenem which showed no resistance (Figure 10).

Presence of genes encoding the production of β-lactamases and toxins genes among identified E. coli

The blaTEM gene was the most observed (51.43%) followed by blaSHV gene (37.14%). Genes encoding blaTEM, blaSHV and blaCTX-M were observed in the E. coli isolates at respective proportions of 17.14, 8.57 and 8.57%. Only one strain of K. pneumoniae carried the blaOXA-1 gene. blaCTX-M was only present in E. coli.
strains. The only isolated Acinetobacter baumannii carried only blaSHV gene (Table 4). No strain of E. coli isolated carried genes coding for the production of shiga-toxins.

**DISCUSSION**

The observation of the sites investigated during our study, indicate insolubility of certain sites with garbage and open gutters wastewater. Some sellers do not have adequate garbage collection bins. Fermented dairy products were displayed on makeshift tables. Inadequate garbage bins were observed with sellers for garbage collection. This increase the attraction of flies, which are not only indicators of poor hygiene, but also vectors of fecal contamination germs (Samapundo et al., 2016). Dairy products are prepared at home and the rest of the activities carried out in the context of the sale is done at the point of sale, using the water available at that site. Most female vendors rarely change rinsing utensils water. No sellers wear masks and gloves, so the hands of some vendors are in direct contact with the money and sold food. These practices increase the possibility of cross-contamination among the vended products. All this is detrimental to good practices in the preparation and sale of these desserts. Previous studies on street foods in Benin made similar observations (Moussé et al., 2016). The microbiological quality of fermented dairy products reveals the presence of fecal coliforms and thermotolerant Gram-negative bacilli isolates including E. coli. This found illustrates a failure of hygiene and implementation of Good Manufacturing Practices (GMP) recorded during the survey. Kouame-Sina et al. (2010) did similar report on the contamination occurs from hands, sanitation of vendors as well as the environment, the water used in the production. Also, according to Zelalem and Bernard (2006), high level of contamination of milk products might be explain by initial contamination originating from the udder surface, washed water, milking materials and utensils used for filtering the milk.

Thus, during our study, among the thermo-tolerant Gram-negative bacilli isolates, 38.9% were K. pneumoniae and 22.2% were E. coli. For E. coli, this rate is lower than the 38% reported by Bagré et al. (2014) on raw milk. The presence of different strain of E. coli gives a good indication of fecal pollution and contamination of milk products which lead to gastroenteritis and food poisoning in human (Galal et al., 2013). Presence of E. coli in milk products constitute a public health hazard. This contamination by E. coli could be explained also by the low hygiene level including handlers’ hand, quality of water used and the used utensils during the processing and dairy products sale. The exposure of dairy products for sale in bowls and packet can be a source of contamination (Zagare et al., 2012). Similar results were early reported on fermented milks in Burkina Faso (Barro et al., 2002; Koussou et al., 2007; Savadogo et al., 2010). In our study dégué, couscous and dégué millet samples were more contaminated in the morning (23.08%) than in the afternoon (19.23%) with respective proportions of 23.08 and 19.23%. The yoghurt samples were only contaminated at 15.38% in the morning. This could be explained by the fact that the initial contamination will be affected by the lowering of pH and the antimicrobial activities of lactic acid bacteria present by the production of bacteriocines (Savadogo et al., 2004; Labioui et al., 2005). Moreover, their decrease may be due to inappropriate conditions for their development such as temperature (Le Conte and Navajas, 2008). The formation of biofilm by strains of thermo-tolerant Gram-negative bacilli, K. pneumoniae displays highest rate of 38.9% followed by the strains E. coli with a proportion of 22.2%. Samples of dégué millet contained more biofilm-forming thermo-tolerant Gram-negative bacilli (34.62%) strains. The formation of biofilm by food-borne thermo-tolerant Gram-negative bacilli strains (especially dairy products) is very serious for human, especially for children. Biofilms are reported to be involved in both device associated infections and tissue infections such as pneumonia and osteomyelitis (Costerton et al., 1999). The study of the sensitivity to antibiotics of the strains of isolated thermo-tolerant Gram-negative bacilli showed the existence of variable resistance proportions according to the antibiotics families. Indeed, against the 16 antibiotics tested, the strains of thermo-tolerant Gram-negative Bacilli show a resistance rate of 92.3% to

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**Table 4.** Proportions of presence of genes coding for the production of β-lactamases.

| Genes       | BlaTEM (%) | BlaSHV (%) | BlaCTX-M (%) | BlaOXA-1 (%) |
|-------------|------------|------------|--------------|--------------|
| Enterobacter cloacae | 3 (8.58%) | 2 (5.71%)  | 0 (0%)       | 0 (0%)       |
| Enterobacter aerogenes | 2 (5.71%) | 1 (2.86%)  | 0 (0%)       | 0 (0%)       |
| Acinetobacter baumannii | 0 (0%)    | 1 (2.86%)  | 0 (0%)       | 0 (0%)       |
| Citrobacter sp         | 1 (2.86%) | 0 (0%)     | 0 (0%)       | 0 (0%)       |
| K. ornithinolytica     | 1 (2.86%) | 1 (2.86%)  | 0 (0%)       | 0 (0%)       |
| K. pneumoniae          | 5 (14.28%)| 5 (14.28%) | 0 (0%)       | 1 (2.86%)    |
| Escherichia coli       | 6 (17.14%)| 3 (8.57%)  | 3 (8.57%)    | 0 (0%)       |
| **Total**              | **18 (51.43%)** | **13 (37.14%)** | **3 (8.57%)** | **1 (2.86%)** |
amoxicillin and to doxycyclin, a resistance rate of 61.5% to ticarcillin and 57.7% resistance to amoxicillin+ clavulanic acid. These found are similar to those of Bagré et al. (2014) on raw and curdled milk sold in Ouagadougou who found a high rate of resistance of E. coli strains to amoxicillin (78.26%) and the highest rate of resistance to amoxicillin+ clavulanic acid (100%). Our data is similar to those of Virpari et al. (2013) which observed the highest resistance rate to Ampicillin (43.75%). This high resistance level could be explained by the abusive and uncontrolled use of antibiotics both in medicine and agriculture, especially in developing countries. This abusive use could induce the acquisition of antibiotic resistance factors by microorganisms (Okeke et al., 1999; Lesch et al., 2001; Trivedi et al., 2011), only E. coli (7.69%) and Enterobacter aerogenes (3.85%) strains produced ESBL. These rates are lower than the 16% observed by Lonchel et al. (2012) in Cameroon on clinical strains. The difference observed can be explained by the origin of the strains involved. Indeed, the strains of clinical origin acquired resistance to antibiotics because they are currently in contact with molecule other than food strains. The observed resistance is due to the abusive and uncontrolled antibiotics use. On the other hand, our strains come from food materials that are supposed to not yet been confronted with the abusive antibiotics use. The only sensitivity was observed with imipenem, which showed a resistance rate of 00%. This suggests that imipenem is still a treatment of choice against the Gram-negative bacilli. During our research, E. coli strains carriers’ blaCTX-M gene and had the highest level of blaTEM. Studies in the food sector have observed the presence of blaSHV, blaTEM and blaCTX-M genes in E. coli strains at high levels. These are the studies conducted by Saad et al. (2019) on chickens in Egypt and that carried out in Iran by Dallal et al. (2018). These studies have shown that the more the strains develop a multidrug resistance to antibiotics the more they are carriers of resistance genes. In our research, we observed the presence of two multi-resistant E. coli strains that are both biofilm-forming, ESBL-producing and carriers of blaCTX-M, blaTEM and blaSHV genes. This state of affairs is very bad for the health of consuming populations.

Conclusion

It appears from our study that artisanal fermented dairy products sold in some secondary schools of Abomey-Calavi and Cotonou are contaminated with thermostolerant Gram-negative bacilli. They have capabilities for biofilm formation, penicillinase production and ESBL production. Resistance strains observed in food are a reality. Awareness raising and training of sellers on good hygiene and manufacturing practices for fermented milk products are necessary to reduce bacterial contamination in order to increase the safety of these products and thus preserve the health of young consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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