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# *Lacticaseibacillus paracasei* is a promising contender for fighting multi-drug-resistant/extensively-drug-resistant *Enterobacteriaceae* and *Pseudomonas* in foods

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**Background**
Multi-drug-resistant/extensively-drug-resistant (MDR/XDR) *Enterobacteriaceae* and other Gram-negative bacteria are among the most important contemporary crises that menace mankind. Their spread into ready-to-eat (RTE) foods is a serious challenge to disease control and is associated with increased morbidity, mortality, and costs to society. It is vital to create non-antibiotic techniques to reduce/mitigate the risk of these microorganisms.

**Objective**
The current study was planned to search for the good candidate(s) biologically active lactic acid bacterial strain(s) to combat MDR/XDR Gram-negative bacteria derived from ready-to-eat foods.

**Materials and methods**
This study was attentive to the isolation of MDR/XDR *Enterobacteriaceae* and other Gram-negative bacteria in various RTE foods. Proteomic, molecular identification, and phylogenetic analysis were carried out on MDR/XDR isolates and biologically active LAB strains; antibiotic resistance profile of MDR *Enterobacteriaceae* and *Pseudomonas* were bent on. Minimum inhibitory (MIP) and bactericidal percentages (MBP) of the 4 supreme *Lacticaseibacillus paracasei'*cell-free supernatants (CFSs) were determined against selected MDR/XDR *Enterobacteriaceae* and *Pseudomonas*. The effects of catalase, pH neutralization, and heat treatment on CFSs' antibacterial activity, and their mode of action using transmission electron microscopy (TEM) were investigated.

**Results and conclusion**
MDR *Enterobacteriaceae* were recovered from all cheese and vegetarian salads, 20 and 33.33% of sausages and luncheon samples on violet red bile glucose agar buttressed with ampicillin, penicillin, and erythromycin, in the presence or absence of tetracycline at 20 µl $ml^{-1}$. Matching to 16S rRNA sequence analysis, the MALDI-TOF MS method successfully identified 48 of 53 *Enterobacteriaceae* isolates to species level. More than 94% of 75 isolated strains were XDR, with MAR indices of up to 0.91; 13.33% were extended-spectrum β-lactamase producers. Of the 67 putative lactic acid bacteria isolates, 23 had inconsistent inhibitory potency; among them, only four isolates were good candidates identified as *Lacticasibacillus paracasei*, their cell-free supernatants (CFSs) were effective against all MDR/XDR *Enterobacteriaceae* and *Pseudomonas* strains with inhibition zones up to 25.00 mm; the CFSs sustained most of their antibacterial activity after treatment with catalase or heating but lost at pH 7. MIP and MBP ranged from 2 to 7% and 3 to 30%, respectively; TEM validated their bactericidal action.

**Keywords:** Antibacterial; multi-drug-resistant / extensively-drug-resistant; *Enterobacteriaceae*; *Pseudomonas; Lacticaseibacillus paracasei;* mode of action

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## Introduction

The discovery of antibiotics is considered one of humanity's sizable findings by no means of the twentieth century. From that time, antibiotics succeeded in human and animal therapy; antibiotics saved countless lives. Regrettably, their effectiveness has been seized over time due to the advent and spread of drug resistance among bacterial pathogens due to inappropriate use or misapplication in different sectors. The sizeable increment of bacterial resistance portends a grave danger; such drawback has become more

exacerbated by increasing the multiplicity of resistance to antibiotics and the limitation of the discovery rate of new ones [1-4].

Freshly, the World Health Organization, WHO [5] updated the antibiotic-resistant pathogens list according to their priority for research and development for public health measures, including 15 families. It grouped them according to their risk into three categories: critical, high, and medium. *Enterobacteriaceae* and other Gram-negative bacteria were at the top of the list, representing the critical category due to their resistance to the latest resort antibiotics, carbapenem and third-generation cephalosporin. However, *Salmonella* Typhi and non-typhoidal *Salmonella* that resist fluoroquinolone fall within a high group [5-7].

*Enterobacteriaceae* is the only family that belongs to the monotypic order, Enterobacterales; such a family inhabits the intestinal tract of vertebrates and other distinct ecological niches. It has large diverse genera and species and has various physiological features. Although it constitutes a normal part of the human and animal gut microbiota, it is incriminated in most infectious diseases worldwide, causing community-acquired and hospital-acquired infections [4, 7].

Taking into consideration the global economic problem, especially in developing countries, poor health conditions, ignorance of the danger of drug-resistant bacteria, and easy access to antibiotics and their indiscriminate use are contemplated among the most important factors that heighten the problematic [21]. Of actual apprehension, is the lack of geographic boundaries to curtail the broadcasting of antibiotic-resistant bacteria. Traveling to high-prevalence countries and antibiotic use are the principal menace factors for new colonization with MDR *Enterobacteriaceae* among the healthy populace; the travelers may become vector-transition when they return to their home countries [22]. Consequently, the advent of MDR bacteria in the food chain, pathogens, and opportunistic pathogens, is nearly unavoidable and threatens the equally developed and developing world. Such bacteria that can reach humans through the ingestion of polluted food pose a serious peril to public health and remain a dare despite enduring progress in the medical field. Ready-to-eat (RTE) foods, whether their ingredients are of plant or animal origin, are among the most important routes of transmission of MDR bacteria; as such foods are consumed without heat processing (8, 23-27].

Given the increasing rates of bacterial resistance to antibiotics, which represents a challenge for control, it is necessary to develop alternative strategies other than antibiotics to reduce/mitigate the risk of these bacteria. These alternatives should be safer for humans and livestock and effective against pathogenic microbes. Some studies have indicated that many natural compounds can help overcome antibiotic resistance. Lactic acid bacteria (LAB) are a significant group of beneficial microorganisms recognized since antiquity and continue to play a vital role in modern daily life. Such a group includes several genera and species with various important biological activities that make them of industrial and health importance [28 -30]. They are generally recognized as safe (GRAS); recent limited studies have proven their capabilities of inhibiting MDR opportunistic and pathogenic bacteria due to their ability to produce a wide range of biomolecules during their growth and fermentation [31 -33]. Most LAB are frequently used as probiotics; and recently have been used as one of the main proposed actions aiming to decolonize the MDR-*Enterobacteriaceae*-gut haulers [20].

In light of the above, the current study was designed to interrogate how much MDR bacteria spread in ready-to-eat foods. Then, it inquired about biologically active strains of LAB bacteria, as good candidate strains, that have antibacterial activity against MDR bacteria, in particular those producing extended-spectrum β-lactamase and those resistant to the latest resort antibiotics, carbapenems, third-generation cephalosporin, and colistin.

## Materials and methods

### Samples assemblage

Forty-seven miscellaneous ready-to-eat food samples, namely vegetarian salad (7), luncheon meat (15), light salt Domiati cheese (5), fermented milk (15 Rayeb), and fermented sausage (5), were arbitrarily gathered in sterile containers from varied marques and different native marketplaces in Giza governorate, Egypt, during 2021. Samples were conveyed swiftly to the laboratory after collecting under cooling conditions.

### Microbiological examination

Once the samples arrived at the laboratory, they were prepared for microbiological examination. Twenty-five grams of each solid sample were aseptically homogenized with 225 ml of saline (0.85 % NaCl) for seconds by blending at high speed; fermented milk samples were homogenized by shaking 25 times. From each homogenate, serial decimal dilutions were prepared up to $10^{-6}$ in the same diluent; at that time, one ml of each dilution was aseptically transferred to inoculate appropriate media in triplicate; the numbers of bacteria were estimated as CFU $g^{-1}$ or CFU $ml^{-1}$. Total aerobic bacteria were determined on nutrient agar (HiMedia Laboratories, Mumbai, India); purple halos (bile precipitation) colonies of *Enterobacteriaceae* were enumerated on violet red bile glucose agar (VRBG, Oxoid). Inoculated plates were incubated in that sequence at 30 ºC or 37 ºC for 48 h. To detect the MDR bacteria, three/four antibiotics were selected as representatives of important classes of antibiotics: B-lactams (penicillin and ampicillin),

macrolide (erythromycin) in the absence or presence of tetracycline (tetracycline). Twenty µg $ml^{-1}$ filter sterilized (Sartorius Stedim Biotech, 0.45 µm) of each antibiotic was added with nutrient and VRBG agar medium; plates were incubated under the pre-described conditions. Lactic acid bacteria (LAB) were detected on De Man-Rogosa-Sharpe (MRS, Merk) and M17 (TM Media) agar media + 0.5% cysteine. The inoculated plates were incubated anaerobically in jars with AnaeroGen (Oxoid) at 37 °C for 48 h.

**Isolation, purification, and identification of multi-drug-resistant (MDR) *Enterobacteriaceae* and other Gram-negative bacteria**

Haphazard take-up was executed for the discrete MDR colonies grown on nutrient and VRBG agar medium buttressed with a combination of antibiotics (20 µg $ml^{-1}$ filter sterilized of each of penicillin, ampicillin, and erythromycin with or sans tetracycline). Isolation and purification were achieved on nutrient agar containing the same multidrug combination. A tally of 148 purified MDR bacterial isolates were initially subjected to preliminary well-known examinations: morphology, Gram staining, oxidase, and fermentation tests. *Enterobacteriaceae* members are short rods, Gram-negative, fermentative, and oxidase-negative. Subsequently, the putative *Enterobacteriaceae* and other Gram-negative isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, VITEK®MS, database version 3, BioMerieux, France). *E. coli* ATCC 8739 was inoculated on the calibration spots as a standardization and interior identification control. Ensuing the manufacturer's guidance, the obtained results were elucidated; the spectral peaks were analogized to the typical spectrum for a species, genus, or family of microorganisms for isolate identification. VITEK® 2 SYSTEM was used to identify only one isolate that could not be identified by MALDI-TOF MS.

**Molecular identification**

Molecular identification was performed to confirm the MALDI-TOF MS and VITEK® 2 SYSTEM identification.

**Genomic DNA extraction from bacterial isolates**

A tally of 75 multi-drug-resistant isolates were grown on Luria-Bertani broth (LB) for 24 hr, then harvested by centrifugation at 12000 g for 5 min. The obtained pellets were washed three times using phosphate buffer. Genomic DNA was extracted using QIAamp® DNA Mini Kit (Cat. No. 51304) per the manufacturer's protocol. DNA concentration and purity were scrutinized by agarose gel electrophoresis and ethidium bromide staining under UV light and NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Germany). The DNA was well-kept at −20 °C.

**Box-PCR fingerprints for genomic typing**

Box-PCR fingerprints were implemented for 75 MDR *Enterobacteriaceae* and other Gram-negative isolates using BOXA1R primer (CTACGGCAAGGCGA CGCTGACG) as annotated by Rademaker and De Bruijn [34]. The PCR reaction was exposed to 34 cycles of amplification: initial denaturation at 94 °C for 7 min, denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 65 °C for 8 min, then succeeded by a final extension of 65 °C for 16 min. 1.5% agarose gel electrophoresis in 0.5 X TBE buffer was used to detach ten µl of the PCR products for 4 h (50 V). BOX-PCR fingerprint band figures were inspected, scrutinized, and collated using the GelJ software v.2.0. Pearson's correlation coefficients and unweighted pair group method average (UPGMA) algorithm were carried out to perform cluster analysis.

**Molecular identification of multi-drug-resistant (MDR) isolates by 16S rRNA gene sequencing**

The 16S rRNA gene of 31 MDR isolates was amplified using the universal primer F-27 (5′-AGAGTTTGAT CMTGGCTCAG- 3′) and R1494 (5′-CTACGGYTACC TTGTTACGAC-3′) according to Lane [35]. PCR reaction was performed using a thermal cycler PCR machine (Bio-rad T100 thermal cycler). The PCR reaction was subjected to 30 cycles of amplification as follows: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, modify the annealing temperature to 58 °C for 1 min, extension at 72 °C for 2 min, then a final extension of 72 °C for 10 min. PCR products were envisaged by agarose gel electrophoresis and then partial 16s rRNA genes were sequenced by Macrogen (Seoul, Republic of Korea). BLASTn (http:// blast. ncbi. nlm. nih. gov/ Blast. cgi) was done to audit the incomplete sequences of 16S rRNA genes and look for their parallel smack in the GenBank database. The 16s rRNA gene sequences of MDR isolates were put down in the NCBI GenBank database under the accession numbers PQ151184 to PQ151214.

**Phylogenetic analysis of the multi-drug-resistant isolates**

Molecular Evolutionary Genetics Analysis (MEGA), version 6 [36] was used to construct the phylogenetic tree. The phylogeny was computed using the maximum composite likelihood method. The phylogenetic tree involved the nucleotide sequences of 31 16S rRNA genes of the MDR

isolates. It involved 30 sequences constituting the closest hits obtained from the NCBI GenBank database.

**Antibiotic susceptibility profile of multi-drug-resistant (MDR) *Enterobacteriaceae* and *Pseudomonas* strains**

The resistotyping of MDR *Enterobacteriaceae* and *Pseudomonas* strains to extra antibiotics belonging to different classes was dictated by employing the disk-diffusion method in Mueller-Hinton (MH) agar as endorsed by the Clinical Laboratory Standards Institute [CLSI, 37]. The tested antibiotics (Bioanalyse) were namely cefotaxime (CTX, 30 µg), meropenem (MER, 10 µg), augmentin (amoxicillin, 20 µg/clavulanic acid, 10 µg, (AMC)), polymyxin B (PMB, 300 units), neomycin (NEO, 30 µg), chloramphenicol (CHL, 30 µg) and nalidixic acid (NAL, 30 µg). The turbidity of each broth culture was adjusted to 0.5 McFarland standard using sterile peptone water, and then 1ml was inoculated into melted Mueller–Hinton (MH) agar medium. After the solidification of inoculated plates, antibiotic discs were positioned on the surface, left for 30 min at room temperature to allow the antibiotic to diffuse, and then inverted and incubated for 18 to 24 h at 37°C. Based on the instruction and inhibition zone diameter breakpoints bent on by CLSI [37], the strains were graded as sensitive, intermediate, and resistant.

**Calculation of multiple antibiotic resistance (MAR) index**

Following the formula, *a/b*, of Krumperman [38], the MAR index was figured by dividing the number of antibiotics to which each isolate was resistant (a) by the total number of antibiotics tested (b).

**Detection of multi-drug-resistant (MDR) *Enterobacteriaceae* strains producing extended-spectrum B-lactamase (ESBL)**

A double-disc synergy test was applied on isolates that showed resistance (R) or diminished susceptibility (I) to 3$^{rd}$ generation cephalosporins (CTX) for screening ESBL-producing strains. In this test, discs of CTX, 30 µg, and augmentin (amoxicillin, 20 µg /clavulanic acid, 10 µg) are kept 20 mm apart, center to center, on inoculated MH agar plate. A positive result is indicated when the inhibition zones around the CTX discs are augmented in the direction of the disc containing clavulanic acid [37].

**Isolation and preliminary identification of lactic acid bacteria (LAB) strains**

From the highest dilutions, random single colonies were taken from M17, and MRS plates and streaked several times on such media following the previously mentioned conditions. Seventy-eight purified isolates were initially tested for Gram stain, cell morphology, catalase, and oxidase activities, and growth reaction in litmus milk. The pure isolates of catalase-negative, oxidase-negative, and curdled skimmed milk were considered presumptive LAB.

**Antibacterial activity of presumptive lactic acid bacteria (LAB) isolates**

Presumptive LAB isolates were screened for their potential antagonistic activities against *Enterobacteriaceae* and *Pseudomonas* MDR strains by the agar well-diffusion method. For each of the 67 putative LAB isolates, the free cell culture supernatant (CFS) was collected by centrifuging at 3000 rpm for 10 minutes under cooling (Centurion, Scientific Ltd, UK). The supernatants were syringe filtered through a 0.22 µm pore size cellulose acetate. The MH agar plate seeded with each MDR strain was prepared following the previous technique; after solidification, 3 wells of 10 mm diameter were made using a sterile cork borer. Then, 100 µl of each of resulted supernatants was aseptically transferred into the wells (in triplicates); the plates were kept for 2 hr in a refrigerator. Afterward, plates were incubated at 37 $^{o}$C for 24 hr; inhibition zone diameter was recorded.

**Identification of the most potent isolates of lactic acid bacteria (LAB)**

The supreme four LAB isolates that showed the highest activities against all MDR strains, as evidenced by growth curb area diameters (up to 25 mm), were further identified by MALDI-TOF MS and genotypically following the previously described techniques. The 16s rRNA gene sequences were put down in the NCBI GenBank database under the accession numbers PQ191449, PQ191450, PQ191451, and PQ191452 for strains 1R1, 2R1, 1S2, and 4S2, respectively; the phylogenetic tree was also constructed.

**Determination of the minimum inhibitory percentage (MIP) and minimum bactericidal percentage (MBP)**

The MIP and MBP of the CFSs of the 4 supreme *Lacticaseibacillus* cultures were bent following the methodology of Chen et al. [33] with some modifications. Different 20 concentrations (1 -10%, 15%, and 20-100%) for each CFS were prepared using sterile MH broth as a diluent. Eight strains of MDR/XDR namely: *Ent. cloacae* 4C4N, *Ent. hormaechei* 1S10V, *E. coli* 6C5V, *Klebsiella pneumoniae* 3S10V, *E.coli* 5S8V, *Serratia marcescens* 2S7N, *Pseudomonas monteilii* 2Sa4V and *Pseudomonas mohnii* 4L2V were selected

according to the number of antibiotics they resisted, which ranged from 5 to 10. For each MDR/XDR strain and *Lactobacillus* cell-free culture, 66 sterile Eppendorf tubes (2 ml) were arranged in three sets, each representing a replicate. Each tube was loaded with 1000 µl of each concentration (in triplicate), inoculated with 10 µl of each 24 hr old MH MDR/XDR *Enterobacteriaceae* or *Pseudomonas* broth culture (final inoculum size reimburse $10^7$ CFU $ml^{-1}$), and incubated for 24 h at 37 °C. Each set contained 2 tubes of MRS: MH (1: 1) broth, one was inoculated as positive control and the other was not inoculated as negative control. Following incubation, the tubes exhibiting no growth and the lowest concentration were designated as the MIP. All broth dilutions that did not grow were streaked onto MH agar and cultured for 24 to 48 h at 37 °C to calculate MBP. The minimum percentage concentration needed to prevent any discernible growth is known as MIP. The MBP was the lowest concentration that caused the eradication of the tested MDR/XDR strains.

**Effects of catalase, pH neutralization, and heat treatment on the antibacterial activity of lactobacilli cell-free supernatants**

A series of experiments were executed to investigate the effect of catalase, pH neutralization, and heat treatment on the antibacterial activity of the CFS of each *Lacticaseibacillus paracasei* strain as portrayed by Scillato et al. [39]. Test tubes were filled with aliquots of each CFS treated with catalase enzyme (1:1 v/w) at pH 7.0 and kept at 37°C for 2 h to evaluate the catalase's effect. To appraise the consequence of temperature, test tubes were filled with 5 ml from each CFS and then heated to 80 for 10 and 30 min, 100, and 121 °C for 20, and 15 min. Each CFS was neutralized to pH 7.0 with 1N NaOH and distributed in test tubes at 5 ml volume. Every test was conducted in triplicate. The antimicrobial activity of each treatment and its control were tested against the selected eight strains of MDR/XDR by applying a well-diffusion method.

**Transmission Electron Microscopy (TEM)**

Transmission electron microscopy (TEM), applying Bermúdez-Puga et al. [40] method, was used to detect the morphological and microstructural commutations of MDR/XDR *E. coli* 6C5V and *Pseudomonas mohnii* 4L2V cells caused by the antagonistic action of cell-free supernatants of *Lacticaseibacillus paracasei* strains 1R1, 2R1, 2S2 and 4S2. Filter-sterilized supernatant of each strain was obtained as pre-described; 24-hr-old cultures of each MDR/XDR strain were used to inoculate filter-sterilized supernatant at a concentration of 1.5 X MIP (final inoculation volume equals $10^7$ CFU $ml^{-1}$) and incubated for 16 hr at 37 °C. MDR/XDR strains were cultured overnight at 37 °C in MH and MRS (1:1) broth and used as a control. Then, the control and treated cultures were centrifuged under cooling at 3000 rpm for 30 min; the resultant pellets were TEM processed for imaging.

**Upkeep of pure cultures**

For routine work, MDR purified isolates were stored at 4˚C for 4 weeks on nutrient agar buttressed with a combination of 3/4 antibiotics, then subcultured. MRS broth cultures of the purified *Lacticaseibacillus paracasei* strains were preserved at 4˚C and re-energized once a week. For prolonged storage, the purified *Lacticaseibacillus* and MDR *Enterobacteriaceae/Pseudomonas* strains, respectively, were kept in MRS broth and LB broth (Miller, Merck) buttressed with a combination of 4 antibiotics at −20 °C with 40% glycerol (v/v).

**Statistics**

ANOVA was used to examine the variations across experimental groups and the data were displayed as mean ± standard deviation. If $p < 0.05$, the difference was considered significant. Prism program version 8 (GraphPad Software, San Diego, CA, USA) was used. For the analysis of the antagonistic effect of LAB isolates' cell-free supernatants against MDR and XDR *Enterobacteriaceae* and *Pseudomonas* strains, a randomized full-block design with three factors, and three replications for each parameter was used. The treatment means were compared using the Assistat program [41] and the Snedecor and Cochran [42] least significant difference (L.S.D.) test.

## Results

**Bacterial load of ready-to-eat foods**

Table 1 and Fig. 1 present an overview of the bacterial load of the examined ready-to-eat food samples, including total aerobic bacteria and *Enterobacteriaceae* counts that were recovered on nutrient and VRBG agar media, as well as the multi-drug resistant bacteria that grew nicely on these media buttressed with a combination of ampicillin, penicillin, erythromycin, either with or without tetracycline at 20 µl-1 of each. The mean values (log10 CFU g-1) of total aerobics for cheese (n=5), luncheon meat (n=15), fermented sausage (n =5) and vegetarian salad (n=7) were 7.04 ± 1.18, 6.49 ± 1.10, 5.09 ± 0.62 and 6.56 ± 0.86, in sequence, Enterobacteriaceae was recouped from all analyzed food samples in comparatively high numbers which occasionally reached 6.33 and 8.11 log10 CFU g-1 with abutting mean values of 5.34 ± 0.85, 5.45 ± 1.63 for salad and cheese; which were significantly higher than those of luncheon and sausage, which had 2.38 ± 0.98, 3.6 ± 0.24 log10 CFU g-1, in sequence. Where there were no statistically significant differences between the total aerobics and the Enterobacteriaceae counts in the

cheese and sausage samples, the differences were significant ($P < 0.05$) for salad and highly significant ($P < 0.00001$) for luncheon meat samples (Fig. 1).

**Table 1** Mean and range values of total aerobic counts, *Enterobacteriaceae*, and multi-drug-resistant bacterial populations grew on media* containing a combination of unrelated antibiotics of examined ready-to-eat food samples ($log_{10}$ CFU $g^{-1}$ or $ml^{-1}$).

| RTE food items | Bacterial load | No. of analyzed samples | No. of +ve samples | Viable count log10 CFU $g^{-1}$ or $ml^{-1}$ | | |
|---|---|---|---|---|---|---|
| | | | | Minimum | Maximum | Mean ± SD |
| Cheese | Total aerobic counts | 5 | 5/5 | 5.88 | 8.36 | 7.04[a] ± 1.18 |
| | [4abs]Total MDR aerobic counts | 5 | 5/5 | 3.0 | 6.24 | 4.10 ±1.25 |
| | *Enterobacteriaceae* | 5 | 5/5 | 4.14 | 8.11 | 5.45 ± 1.63 |
| | [4Abs]MDR *Enterobacteriaceae* | 5 | 5/5 | 1.70 | 6.10 | 3.52 ± 1.63 |
| Luncheon | Total aerobic counts | 15 | 15/15 | 5.03 | 9.0 | 6.49 ± 1.10 |
| | [3Abs]Total MDR aerobic counts | 15 | 15/15 | 2.97 | 7.36 | 4.24 ± 1.20 |
| | [4Abs]Total MDR aerobic counts | 15 | 0/15 | - | - | - |
| | *Enterobacteriaceae* | 15 | 15/15 | 1.00 | 4.29 | 2.38 ± 0.98 |
| | [3Abs]MDR *Enterobacteriaceae* | 15 | 5/15 | 0.0 | 3.67 | 0.71 ± 1.19 |
| | [4Abs]MDR *Enterobacteriaceae* | 15 | 0/15 | - | - | - |
| Fermented sausage | Total aerobic counts | 5 | 5/5 | 4.08 | 5.68 | 5.09 ± 0.62 |
| | [3Abs]Total MDR aerobic counts | 5 | 5/5 | 3.45 | 4.68 | 4.01 ± 0.50 |
| | [4Abs]Total MDR aerobic counts | 5 | 0/5 | - | - | - |
| | *Enterobacteriaceae* | 5 | 5/5 | 3.23 | 3.86 | 3.60 ± 0.24 |
| | [3Abs] MDR *Enterobacteriaceae* | 5 | 1/5 | 0.0 | 3.51 | 0.70 ± 1.57 |
| | [4Abs]MDR *Enterobacteriaceae* | 5 | 0/5 | - | - | - |
| Vegetarian salad | Total aerobic counts | 7 | 7/7 | 5.46 | 7.56 | 6.56 ± 0.86 |
| | [4Abs]Total MDR aerobic counts | 7 | 7/7 | 3.15 | 5.92 | 4.31 ± 0.89 |
| | *Enterobacteriaceae* | 7 | 7/7 | 4.23 | 6.33 | 5.34 ± 0.85 |
| | [4abs]MDR *Enterobacteriaceae* | 7 | 7/7 | 3.12 | 4.56 | 3.54 ± 0.48 |
| Fermented milk (Rayeb) | Total aerobic counts | 15 | 0/15 | - | - | - |
| | [3Abs]Total MDR aerobic counts | 15 | 0/15 | - | - | - |
| | [4Abs]Total MDR aerobic counts | 15 | 0/15 | - | - | - |
| | *Enterobacteriaceae* | 15 | 0/15 | - | - | - |
| | [3Abs]MDR *Enterobacteriaceae* | 15 | 0/15 | - | - | - |
| | [4abs]MDR *Enterobacteriaceae* | 15 | 0/15 | - | - | - |

* Media used = nutrient agar for total aerobics; VRBG agar for *Enterobacteriaceae.*
3Abs = growth on media buttressed with a combination of penicillin, ampicillin, and erythromycin, 20 μg $ml^{-1}$ of each.
4Abs = growth on media buttressed with a combination of penicillin, ampicillin, tetracycline, and erythromycin, 20 μg $mL^{-1}$ of each.

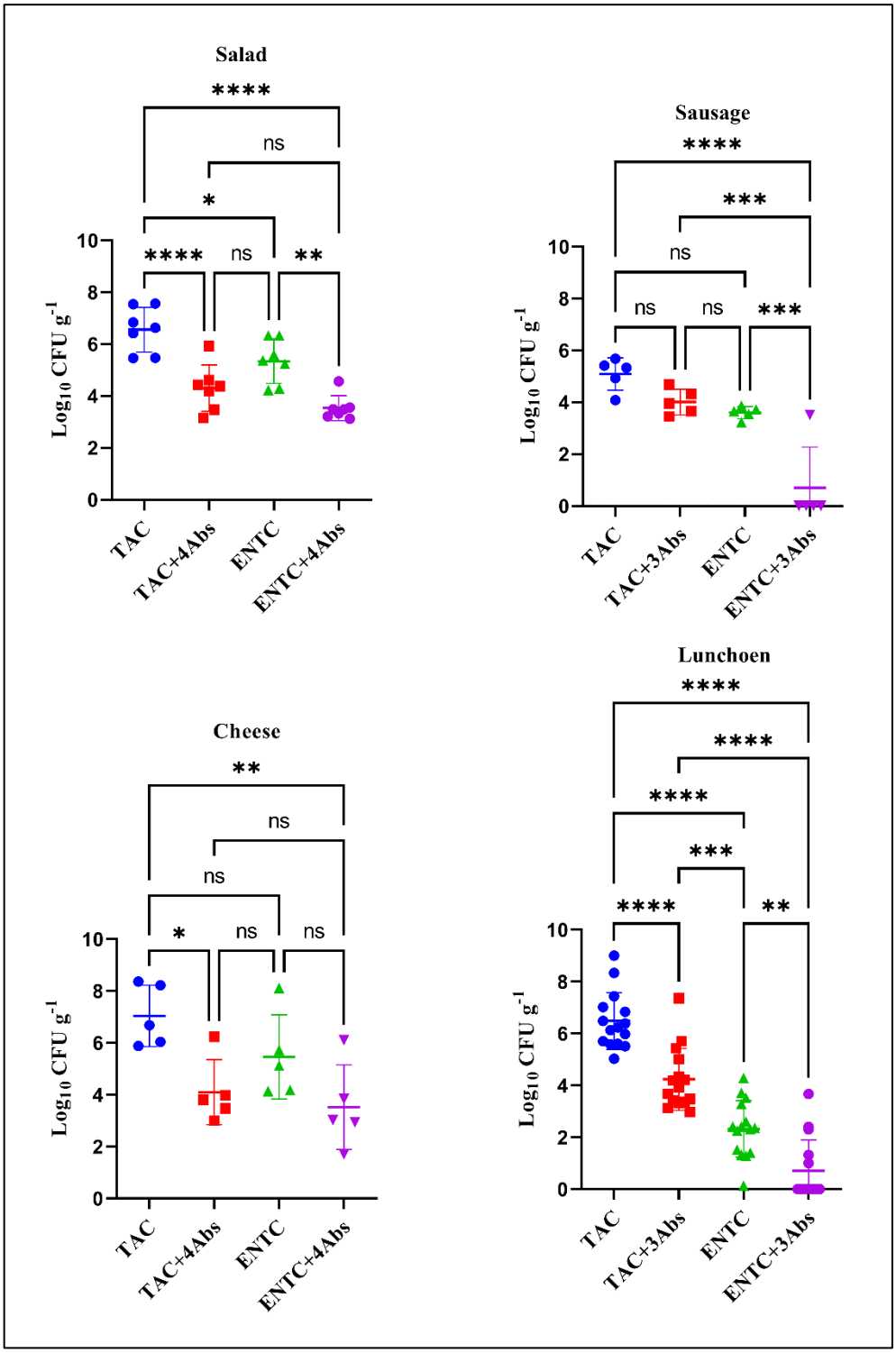



**Fig. 1** Prevalence of MDR bacteria on ready-to-eat food samples, salad (n=7), cheese (n=5), luncheon meat (n=15) and fermented sausage (n=5). Data are expressed as mean (± SD, n = 3). The significance level between pairs is indicated by the number of symbols, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and ns for non-significant. TAC= total aerobic counts; ENTC = *Enterobacteriaceae*; 4Abs= penicillin, ampicillin, tetracycline and erythromycin each of 20 µg $ml^{-1}$; 3Abs = penicillin, ampicillin and erythromycin each of 20 µg $ml^{-1}$

Regarding MDR bacteria, all cheese and salad samples harbored these bacteria, as they were able to grow on nutrient and VRBG agar media buttressed with four irrelevant antibiotics. Their averages were 4.10 ± 1.25 and 4.31 ± 0.89 for total aerobics, 3.52 ± 1.63 and 3.54 ± 0.48 for *Enterobacteriaceae*, in sequence*;* the differences between means were not statistically significant (Fig. 1). Meanwhile, in the absence of tetracycline, the bacterial load of luncheon meat (15 samples) and sausages (5 samples) that failed to grow on media buttressed with the four irrelevant antibiotics, were able to develop on such media, albeit at varying rates. The mean values of total MDR bacteria for all luncheon meat and sausage samples were 4.24 ± 1.20 and 4.01 ± 0.50 $\log_{10}$ CFU $g^{-1}$, respectively (Table 1). On the other hand, MDR *Enterobacteriaceae* were significantly less common than other multidrug-resistant bacteria, appearing in only 5/15 and 1/5 of luncheon meat and sausage samples with comparable averages, respectively (Table 1 and Fig. 1). Whether the media contained the combination of antibiotics in presence or absence of tetracycline, all the 15 samples of fermented milk (Rayeb) were free of total aerobics, *Enterobacteriaceae*, and MDR bacteria.

**Identification, prevalence, and distribution of multi-drug-resistant (MDR) isolates**

Initial identification was performed on 148 pure MDR bacterial isolates from RTE-food samples that were recovered on various media, whether they contained a combination of three or four antibiotics. Table 2 shows that 62.5, 3.92, 5.0, and 65.57% of MDR isolates from cheese, luncheon, sausage, and salad, in that order, were tentatively identified as *Enterobacteriaceae*.

While this study was designed to pivot on MDR *Enterobacteriaceae*, other bacterial populations with analogous phenotypic traits different from those of *Enterobacteriaceae.* While this study was designed to pivot on MDR *Enterobacteriaceae*, other bacterial populations with analogous phenotypic traits different from those of *Enterobacteriaceae* were spread over the agar media containing a combination of unrelated antibiotics. The population in question was frequently found on luncheon and sausage samples, which piqued interest in identifying the bacteria involved (22 MDR isolates).

**Table 2** Preliminary identification of multi-drug-resistant bacterial isolates recovered on nutrient and violet red bile glucose agar media from different ready-to-eat food samples.

| Ready-to-eat foods | No. of isolates | Gram-negative | Oxidase test | | Fermentation test | | % presumptive *Enterobacteriaceae* |
|---|---|---|---|---|---|---|---|
| | | | Negative | Positive | Fermentative | Non-fermentative | |
| **Cheese** | **16** | **14** | **10** | **4** | **10** | **4** | **62.50** |
| **Luncheon** | **51** | **20** | **2** | **18** | **2** | **18** | **3.92** |
| **Sausage** | **20** | **5** | **1** | **4** | **1** | **4** | **5.00** |
| **Salad** | **61** | **53** | **40** | **13** | **40** | **13** | **65.57** |

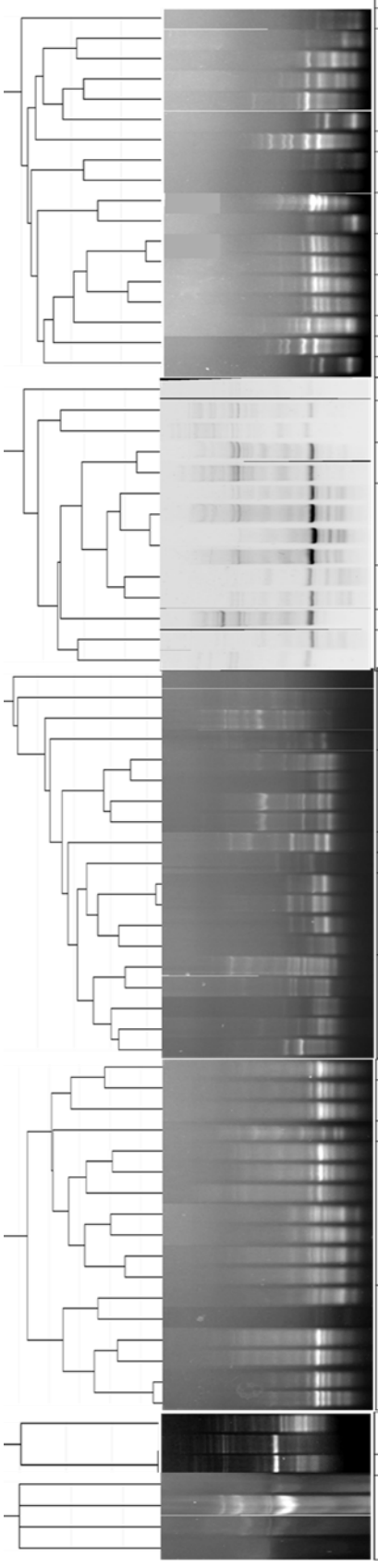
Based on MALDI-TOF mass spectrometry identification, 74 out of 75 (98.67%) MDR isolates were identified as 16 *Escherichia coli* (4 from cheese, 12 from salad); 13 *Klebsiella pneumoniae* (1 from sausage, 12 from salad); 10 *Serratia marcescens* (3 from cheese, 7 from salad); 6 *Enterobacter cloacae* (1 from cheese, 1 from luncheon, 4 from salad); 5 *Enterobacter hormaechei* (2 from cheese, 3 from salad); 2 *Enterobacter kobei* (1 from luncheon, 1from salad). All the 22 MDR isolates of luncheon (18) and sausage (4) were identified as *Pseudomonas putida*. Only one of the seventy-five MDR isolates was not recognized by MALDI-TOF MS; this isolate was identified by VITEK® 2 SYSTEM as *Kluyvera cryocrescens* (Fig. 2). Box-PCR was created for genomic typing for all the 75 MDR strains recouped from cheese, fermented sausage, luncheon, and vegetarian salad. All these strains shaped 19 clusters with two or more shared fingerprints (Fig. 2). Cluster 1 (2S9V, 6S9N, 3S8V, 4C4N, and 4S9V); cluster 2 (1S2V and 1S11V); cluster 3 (5C2V and 6S2V); cluster 4 (1S10V and 4C2N); cluster 5 (2Sa4V and 1L2V); cluster 6 (3S2V and 1C3V); cluster 7 (3S2TN and 12S7V); cluster 8 (6S7V, 9S7V,6C5V, and 2S7V); cluster 9 (3S7V and 4S7V); cluster 10 (8S7V and 1S7N); cluster 11 (1C5V, 1C4N, 2S11V, and 3S11V); cluster 12 (6S11V, 7S11V, 3S11N, and 3S11RN); cluster 13 (5Sa4V, 6S8V, 2S2V, and 4S2V); cluster 14 (6S2RV, 2S10V, 4S10V, 5S10V, and 6S10V); cluster 15 (2Sa4N, 3L3N, and 4Sa3N); cluster 16

(4Sa4V, 1L1V, 2L1V, 5L2V, 6L2V, 4L3RN, and 1L3V); cluster 17 (6L4V, 1L5V, 2L6V, 1L2N, 3L1V, and 4L2V); cluster 18 (5S8V and 5S8RV) and cluster 19 (4S11V, 5S11V, 7S2V, and 3S10V). Eleven isolates (4L1V, 5L1V, 3L2V, 2L2N, 1L3N, 2C1V, 3C3V, 3C1V, 2S7N, 4L4V and 2S11N) exhibited a unique Box-PCR fingerprint profile.

Only one representative isolate from each closely clustered distinct fingerprint profile was identified based on the sequence of the 16S rRNA gene. Out of 31 strains, 23 strains (74.2%) belonged to *Enterobacteriaceae*, in comparison, eight strains (25.80%) were affiliated with *Pseudomonadaceae* (*Ps. putida*) as assigned by MALDI-TOF MS. Beyond *Enterobacteriaceae* strains, eight isolates represented 8 clusters (3S2V, 3S2TN, 3S7V, 8S7V, 5S8RV, 2C1V, 3C3V and 6C5V) showed 100% similarity to *E. coli*; four isolates from 3 clusters (4S2V, 2S10V, 7S2V and 3S10V) presented 100% resemblance to *K. Pneumoniae*; four isolates from 4 clusters (6S11V, 2S7N, 1C5 and 3C1V) were 100% analogous to *S. marcescens*; two isolates from 2 clusters (4C4N and 4L1V) were identified as *Ent. cloacae* with 100% similarity, two isolates from 2 clusters (1S2V, 5L1V) were 100% similar to *Enterobacter* sp., and only one isolate of each (1S10V, 6S2V and 2S11N) was affiliated to *Ent. hormaechei*, *Ent. chuandaensis*, *Kluyvera cryocrescens*, in the respective order (Fig. 2). Among 8 *Pseudomonas* strains, two isolates (4L4V and 4L2V) were identified as *Pseudomonas mohnii*, three isolates were 100% resemble *Pseudomonas* sp., two isolates corresponded to *Pseudomonas monteilii* with 100% similarity and only one isolate was recognized as *Pseudomonas plecoglossicida* with 100% likeness. Accordingly, the 22 *Pseudomonas* strains were identified as (3) *Ps. monteilii*, (7) *Ps. plecoglossicida*, (7) *Ps. mohnii*, and (5) *Pseudomonas* sp. Confirmation of the phylogenetic relatedness was validated through the neighbor-joining tree (Fig. 3a).

**Resistance pattern of Multi-drug-resistant/extensively-drug-resistant (MDR/XDR) *Enterobacteriaceae* and *Pseudomonas* strains isolated from ready-to-eat food samples**

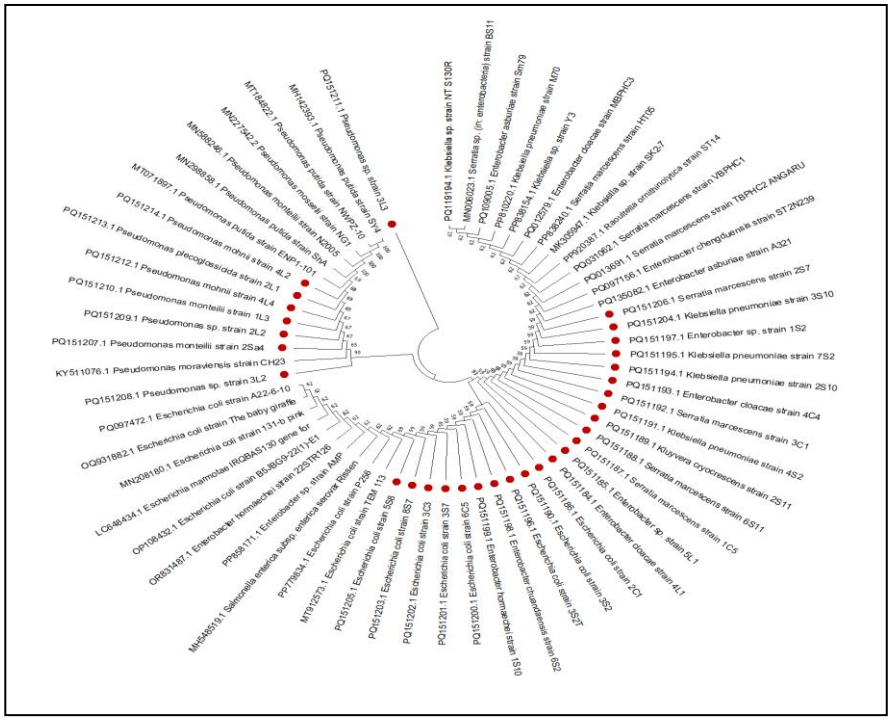
Entirely, *Enterobacteriaceae* strains were resistant to penicillin, ampicillin, tetracycline, and erythromycin. However, all strains were vulnerable to polymyxin B, except one. Most *E. coli strains* showed resistance to cefotaxime (13, 81.25%), neomycin (12, 75%), and chloramphenicol (12, 75%). Only one (6.25%), two (12.50%), and three (18.75%) strains of *E. coli* displayed resistance to meropenem, nalidixic acid, and amoxicillin-clavulanic acid, respectively. Low recurrence of resistance to cefotaxime (23.08%, three strains) and neomycin (15.38%, two strains) compared to chloramphenicol (69.23%, nine strains) was observed with *K. pneumoniae* strains. Concerning *Enterobacter* species, all strains (13) were susceptible to carbapenem (meropenem) except two strains of *Enterobacter* sp. and *Ent. chuandaensis* that were intermediately resistant. Five (83.33%) and three (50%) strains of *Ent. cloacae* were resistant to amoxicillin-clavulanic acid and cefotaxime, in sequence, only one strain (16.67%) showed resistance to both chloramphenicol and nalidixic acid; additionally, these strains revealed either intermediate resistance (33.33%) or sensitivity (66.67%) to neomycin. All the three *Enterobacter* sp. were susceptible to chloramphenicol, and nalidixic acid; two strains showed resistance to cefotaxime and amoxicillin-clavulanic acid, and only one was resistant to neomycin. While one strain of *Ent. hormaechei* was resistant to cefotaxime and the other to neomycin, both strains were resistant to amoxicillin-clavulanic acid and chloramphenicol. One of the *Ent. chuandaensis* resisted cefotaxime and amoxicillin-clavulanic acid; both were resistant to chloramphenicol. The majority of *Serratia marcescens* (90%) were resistant to amoxicillin-clavulanic acid, whereas the lowest frequencies of resistance were for cefotaxime (2 strains, 20%); only one strain of them showed resistance to polymyxin B, neomycin, chloramphenicol, and nalidixic acid. All *Serratia* strains were susceptible (80%) or intermediately resistant (20%) to meropenem. All *Pseudomonas* species were resistant to penicillin, ampicillin, cefotaxime, erythromycin, chloramphenicol, and nalidixic acid; the highest rate of resistance to meropenem was observed in 12 strains (54.54%) of all *Pseudomonas* species as (4) *Ps. mohnii*, (5) *Ps. plecoglossicida*, (1) *Pseudomonas* sp., and (2) *Ps. monteilii* strains. Most *Pseudomonas* species showed intermediate resistance (86.36%) to neomycin, only three strains of *Ps. mohnii* were resistant. In contrast to *Enterobacteriaceae*, only two *Pseudomonas* strains belonging to *Ps. mohnii* were resistant to tetracycline, and the majority were sensitive (Fig. 4 a and b).



| Isolate's code | Isolation source | MALDI-TOF | 16srRNA | Accession number |
|---|---|---|---|---|
| 4L1V | Luncheon | *Enterobacter cloacae* | *Ent. cloacae* | PQ151184 |
| 2S9V | Salad | *Ent. cloacae* | | |
| 6S9N | Salad | *Ent. cloacae* | | |
| 3S8V | Salad | *Ent. cloacae* | | |
| 4C4N | Cheese | *Ent. cloacae* | *Ent. cloacae* | PQ151193 |
| 4S9V | Salad | *Ent. cloacae* | | |
| 5L1V | Luncheon | *Ent. kobei* | *Enterobacter sp.* | PQ151185 |
| 1S2V | Salad | *Ent. kobei* | *Enterobacter sp.* | PQ151197 |
| 1S11V | Salad | *Ent. hormaechei* | | |
| 5C2V | Cheese | *Ent. hormaechei* | | |
| 6S2V | Salad | *Ent. hormaechei* | *Ent. chuandaensis* | PQ151198 |
| 1S10V | Salad | *Ent. hormaechei* | *Ent. hormaechei* | PQ151199 |
| 4C2N | Cheese | *Ent. hormaechei* | | |
| 2Sa4V | Sausage | *Pseudomonas putida* | *Ps. monteilii* | PQ151207 |
| 1L2V | Luncheon | *Ps. putida* | | |
| 3L2V | Luncheon | *Ps. putida* | *Pseudomonas sp.* | PQ151208 |
| 2L2N | Luncheon | *Ps. putida* | *Pseudomonas sp.* | PQ151209 |
| 1L3N | Luncheon | *Ps. putida* | *Ps. monteilii* | PQ151210 |
| 2C1V | Cheese | *Escherichia coli* | *E. coli* | PQ151186 |
| 3S2V | Salad | *E. Coli* | *E. coli* | PQ151190 |
| 1C3V | Cheese | *E. Coli* | | |
| 3S2TN | Salad | *E. Coli* | *E. coli* | PQ151196 |
| 12S7V | Salad | *E. Coli* | | |
| 6S7V | Salad | *E. Coli* | | |
| 9S7V | Salad | *E. Coli* | | |
| 6C5V | Cheese | *E. Coli* | *E. coli* | PQ151200 |
| 2S7V | Salad | *E. Coli* | | |
| 3S7V | Salad | *E. Coli* | *E. coli* | PQ151201 |
| 4S7V | Salad | *E. Coli* | | |
| 3C3V | Cheese | *E. Coli* | *E. coli* | PQ151202 |
| 8S7V | Salad | *E. coli* | *E. coli* | PQ151203 |
| 1S7N | Salad | *E. coli* | | |
| 1C5V | Cheese | *Serratia marcescens* | *S. marcescens* | PQ151187 |
| 1C4N | Cheese | *S. marcescens* | | |
| 2S11V | Salad | *S. marcescens* | | |
| 3S11V | Salad | *S. marcescens* | | |
| 6S11V | Salad | *S. marcescens* | *S. marcescens* | PQ151188 |
| 7S11V | Salad | *S. marcescens* | | |
| 3S11N | Salad | *S. marcescens* | | |
| 3S11RN | Salad | *S. marcescens* | | |
| 3C1V | Cheese | *S. marcescens* | *S. marcescens* | PQ151192 |
| 2S7N | Salad | *S. marcescens* | *S. marcescens* | PQ151206 |
| 5Sa4V | Sausage | *Klebsiella pneumoniae* | | |
| 6S8V | Salad | *K. pneumoniae* | | |
| 2S2V | Salad | *K. pneumoniae* | | |
| 4S2V | Salad | *K. pneumoniae* | *K. pneumoniae* | PQ151191 |
| 6S2RV | Salad | *K. pneumoniae* | | |
| 2S10V | Salad | *K. pneumoniae* | *K. pneumoniae* | PQ151194 |
| 4S10V | Salad | *K. pneumoniae* | | |
| 5S10V | Salad | *K. pneumoniae* | | |
| 6S10V | Salad | *K. pneumoniae* | | |
| 2Sa4N | Sausage | *Ps. putida* | | |
| 3L3N | Luncheon | *Ps. putida* | *Pseudomonas* sp.. | PQ151211 |
| 4Sa3N | Sausage | *Ps. putida* | | |
| 4L4V | Luncheon | *Ps. putida* | *Ps. mohnii* | PQ151212 |
| 4Sa4V | Sausage | *Ps. putida* | | |
| 1L1V | Luncheon | *Ps. putida* | | |
| 2L1V | Luncheon | *Ps. putida* | *P. plecoglossicida* | PQ151213 |
| 5L2V | Luncheon | *Ps. putida* | | |
| 6L2V | Luncheon | *Ps. putida* | | |
| 4L3RN | Luncheon | *Ps. putida* | | |
| 1L3V | Luncheon | *Ps. putida* | | |
| 6L4V | Luncheon | *Ps. putida* | | |
| 1L5V | Luncheon | *Ps. putida* | | |
| 2L6V | Luncheon | *Ps. putida* | | |
| 1L2N | Luncheon | *Ps. putida* | | |
| 3L1V | Luncheon | *Ps. putida* | | |
| 4L2V | Luncheon | *Ps. putida* | *Ps. mohnii* | PQ151214 |
| 5S8V | Salad | *E. coli* | | |
| 5S8RV | Salad | *E. coli* | *E. coli* | PQ151205 |
| 2S11N | Salad | *Kluyvera cryocrescens** | *Klu. cryocrescens* | PQ151189 |
| 4S11V | Salad | *K. pneumoniae* | | |
| 5S11V | Salad | *K. pneumoniae* | | |
| 7S2V | Salad | *K. pneumoniae* | *K. pneumoniae* | PQ151195 |
| 3S10V | Salad | *K. pneumoniae* | *K. pneumoniae* | PQ151204 |

**Fig. 2.** Box-PCR fingerprints of 75 MDR/XDR of *Enterobacteriaceae* and *Pseudomonas* strains recuperated from ready-to-eat food based on the MALDI-TOF MS identification and compared to 16S rRNA sequence analyses. * It was not recognized by MALDI-TOF MS and identified by VITEK® 2 SYSTEM.

**(a)**




**(b)**

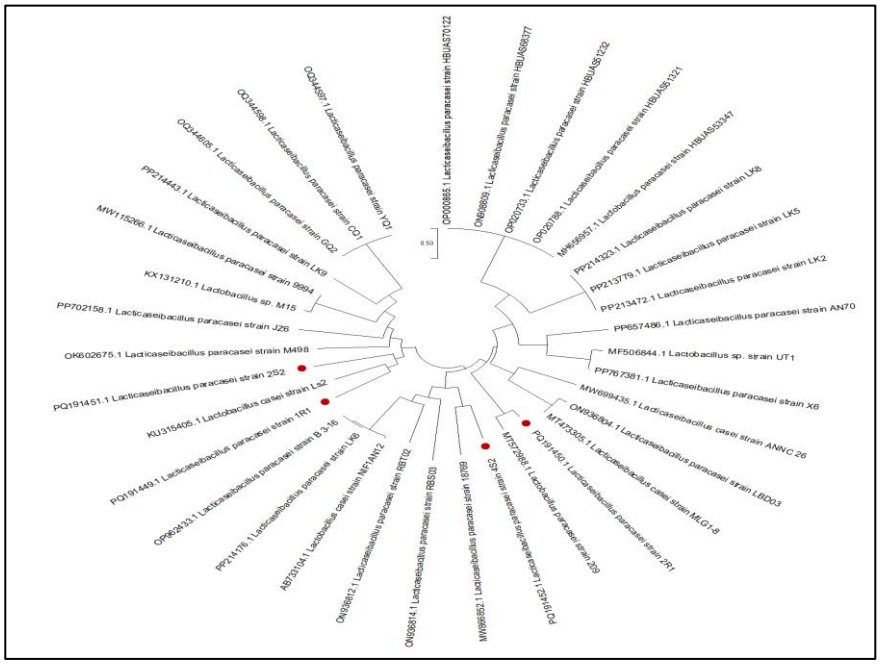



**Fig. 3** A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of: (a), 31 MDR/XDR strains of *Enterobacteriaceae* and *Pseudomonas* (Red circles); (b) the most potent lactic acid bacteria strains (Red circles) with the closest hits obtained from the NCBI Gene Bank.

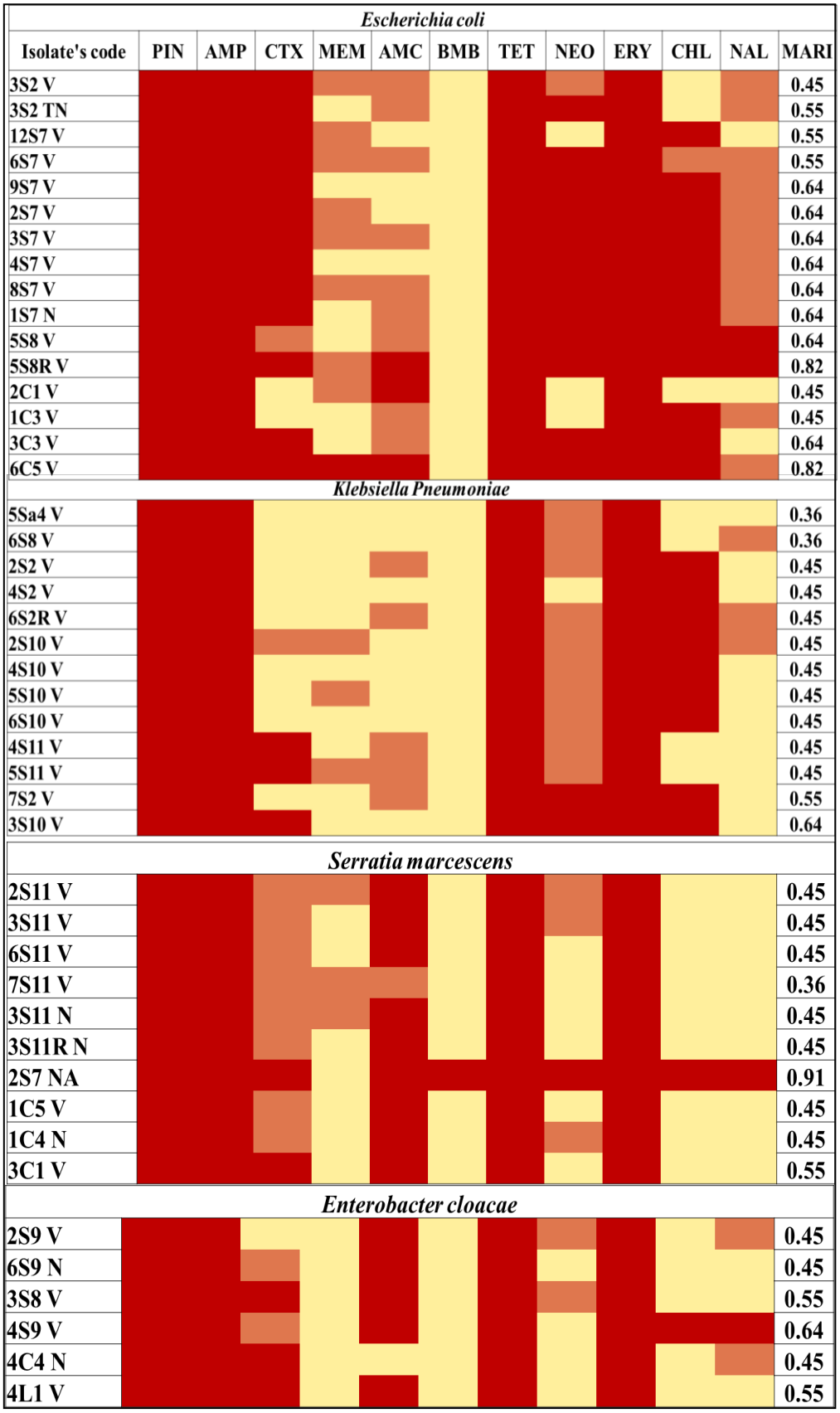



**Fig. 4a** Heat map representing resistance pattern of MDR/XDR *E. coli, K. pneumonia, S. marcescens* and *Ent. cloacae* strains isolated from ready-to-eat food samples. Color coding: red = resistant; orange = intermediate and yellow = sensitive.

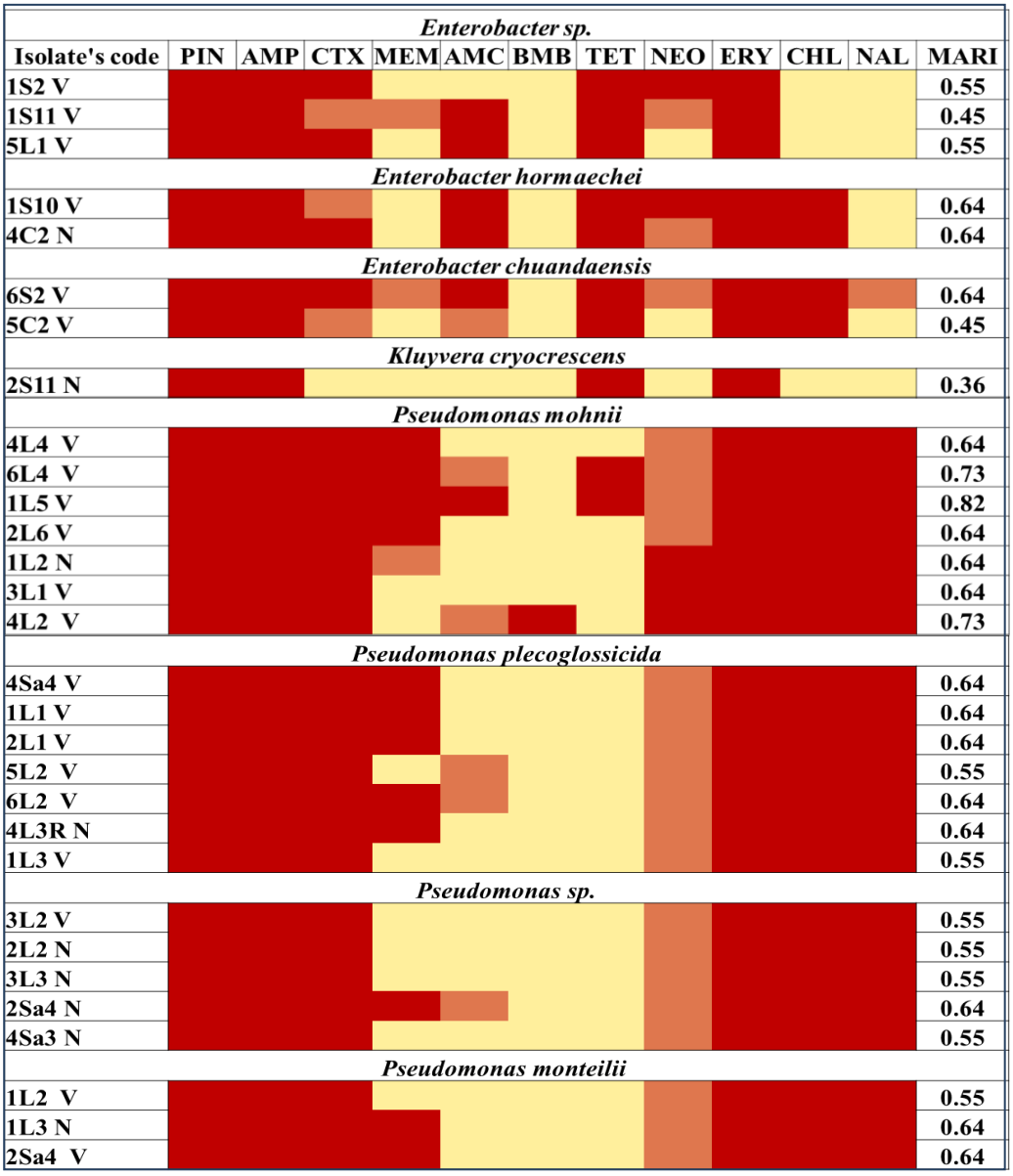



**Fig. 4b.** Heat map representing resistance pattern of MDR/XDR *Enterobacter* sp., *Ent. hormaechei, Ent. chuandaensis, Klu. Cryocrescens, Ps. mohnii. Ps. plecoglossicida, pseudomonas sp.,* and *Ps. monteilii* strains isolated from ready-to-eat food samples. Color coding: red = resistant; orange = intermediate and yellow = sensitive.

Concerning β-lactams, all isolates were resistant to penicillin and ampicillin (20 µg $ml^{-1}$); the third-generation cephalosporins (cefotaxime) demonstrated comparatively good efficacy likened to penicillin and ampicillin. However, the majority of the strains (47 strains, > 60 %) exhibited resistance, and 20 % (15 isolates) displayed an intermediate response. About 83% (39) of these positive strains showed zero inhibition zones, confirming extremely severe resistance. Overall, of the food items examined, 100% of luncheon isolates, 80% of fermented sausage, 50% of cheese, and 45% of vegetarian salads had cefotaxime resistance (Fig. 5a). Meropenem was more successful, albeit only a small percentage of isolates (13 isolates, >17%) showed resistance; of those, 12 *Pseudomonas* strains related to luncheon and sausage accounted for more than 92% of the resistant isolates. More than 30 % (23 strains) showed resistance to augmentin (amoxicillin-clavulanic acid); except for one *Pseudomonas* strain, all remaining strains (29.33%) belonged to

*Enterobacteriaceae*. Of these resistant strains, 26.09 % (6 strains) verified an extreme resistance as indicated by zero inhibition zones. Resistance to the β-lactamase inhibitor (amoxicillin-clavulanic acid) and meropenem was low compared to third-generation cephalosporins (cefotaxime). Polymyxin B was the most effective antibiotic since it is used as a last resort. Just two (2.67%) of the 75 MDR strains exhibited resistance: *Pseudomonas mohnii* and *Serratia marcescens*, which showed extreme resistance as evidenced by zero mm inhibition zone. Compared to *Enterobacteriaceae* strains, tetracycline potency was significantly more effective against *Pseudomonas*. This antibiotic did not demonstrate any impact against all *Enterobacteriaceae* strains under investigation; only two strains of *Ps. mohnii* showed resistance. All *Enterobacteriaceae* and *Pseudomonas* strains were erythromycin-resistant. Neomycin-resistant strains accounted for 26.67% of all MDR strains, with the majority (22.67%) isolated from cheese and salad belonging to the *Enterobacteriaceae* family. Among the 75 MDR strains, 65.33% showed resistance to chloramphenicol; they were derived in the following proportions from salad, luncheon, cheese, and sausage: 29.33 (22 strains), 24.0 (18 strains), 6.67 (5 strains), and 5.33% (4 strains), in that order. Just 10% of MDR strains from salad showed resistance to nalidixic acid, whereas all strains from luncheon and sausage were nalidixic acid-resistant.

More than 94% of the bacterial strains in the current study were extensively drug-resistant (XDR), with MAR indices of up to 0.91 indicating resistance to at least five antibiotic classes (Figs. 4 and 5b).

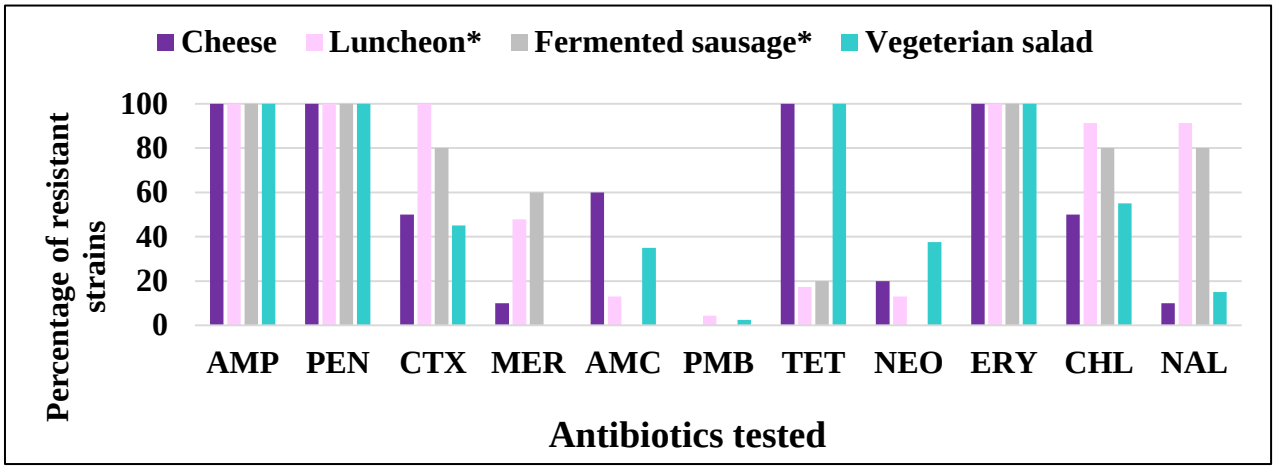
**The frequency of extended-spectrum β-lactamase (ESBL) multi-drug-resistant/extensively-drug-resistant (MDR/XDR) strains**

Out of 75 MDR/XDR food isolates, 10 strains (13.33 %) were extended-spectrum β-lactamase (ESBL) producers (Fig. 5c); the majority (9.33%) were isolated from 3 luncheon samples and belonged to *Pseudomonas* spp. Overall, the ESBL producers were distributed as follows: *Ps. mohnii* (4.00%), *Ps. monteilii* (2.67%), *Ps. plecoglossicida* (1.33%), *Pseudomonas* sp. (2.67) from luncheon; *Ps. monteilii* (1.33%) from sausage and *Enterobacter cloacae* (1.33%) from cheese. As shown in Fig. (5c), all *Pseudomonas* species displayed uniform resistance to ampicillin, penicillin, cefotaxime, erythromycin, chloramphenicol, and nalidixic acid; but differed in resistance to meropenem, polymyxin B and neomycin. All *Ps. mohnii* strains were resistant to neomycin; only one was resistant to polymyxin B; and two strains of *Ps. monteilii* were resistant to meropenem.

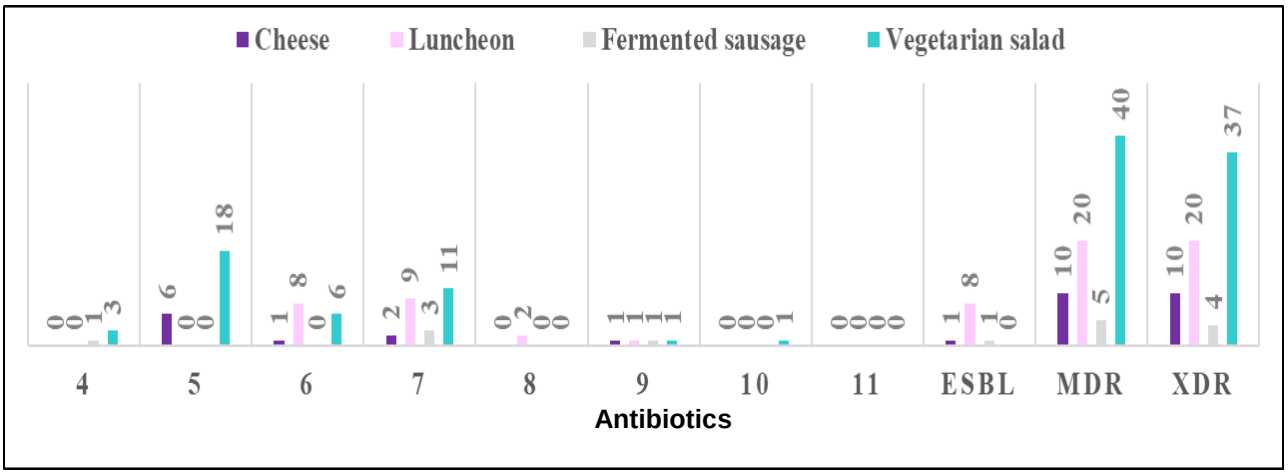
**Identification of lactic acid bacteria strains and *in vitro* antagonistic bioassay against multi-drug-resistant and extensively-drug-resistant *Enterobacteriaceae* and *Pseudomonas* strains**

Fermented milk (Rayeb) and fermented sausage yielded 67 isolates of putative LAB bacteria. Although the growth of such isolates dropped the pH to be less than 4 in most cases, most of them were futile in inhibiting the growth of MDR/XDR strains. Only 23 (34.33%) have a relatively inconsistent inhibition potency against the MDR/XDR strains as shown in Tables (S1-11). Four isolates had antagonistic effects against all the 75 MDR/XDR *Enterobacteriaceae* and *Pseudomonas* strains with inhibition zones ranging from 10.67 to 25.00 mm; the antagonistic activity was species- and strain-dependent. Their morphology was non-spore-forming rod-shaped in pairs or chains. MALDI-TOF MS identified these potent LAB isolates at the genus and species levels. Three were identified as *Lacticaseibacillus paracasei* (2R1, 4S2, and 2S2), and the last one as *Lacticaseibacillus paracasei* (1R1). An accurate identification was obtained from 16S rRNA gene sequence analysis, which indicated that all the sequences of these candidates correspond to *Lacticaseibacillus paracasei* with a similarity of 100%. They were submitted to GenBank under the accession numbers PQ191449, PQ191450, PQ191451, and PQ191452 for strains 1R1, 2R1, 1S2, and 4S2, respectively; phylogenetic relatedness was validated through the neighbor-joining tree (Fig. 3b).

**(a)**




**(b)**




**(c)**

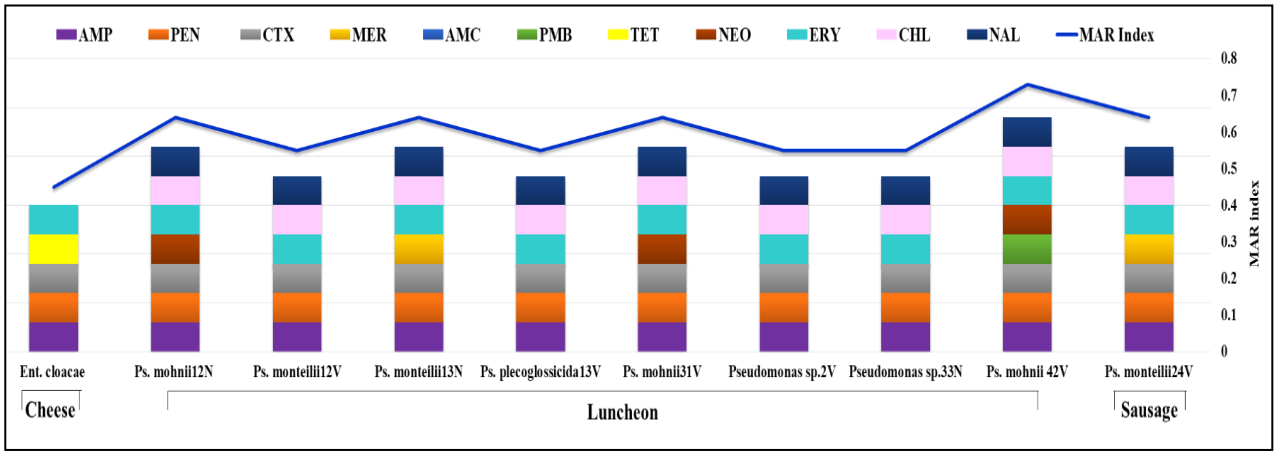



**Fig. 5** Resistance pattern of *Enterobacteriaceae* strains and *Pseudomonas* species isolated from ready-to eat food samples, (a); (b) number of antibiotics to which strains showed resistance, extended spectrum β-lactamase (ESBL) producing strains, multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains; (c) antibiotic resistance patterns of ESBL producing strains, c). Amp, ampicillin; PEN, penicillin; CTX, cefotaxime; MER, meropenem, AMC, augmentin (amoxicillin, 20 µg/clavulanic acid, 10 µg; PMB, polymyxin B; TET, tetracycline; NEO, neomycin; ERY, erythromycin; CHL, chloramphenicol and NAL, nalidixic acid. * 90 and 80% of luncheon and fermented sausage isolates are *Pseudomonas.*

**The minimum inhibitory percentage (MIP) and the minimum bactericidal percentage (MBP)**

CFSs of *Lacticaseibacillus paracasei* strains have proven their greatest potency in inhibiting/killing the growth of tested MDR/XDR *Enterobacteriaceae* and *Pseudomonas* species. Table 3 shows the minimum inhibitory (MIP) and minimum bactericidal percentages (MBP) for the four potent *Lacticaseibacillus* strains against *Enterobacteriaceae* and *Pseudomonas* species. The MIP ranged from 2 to 7%; except *Ps. monteilii* 2Sa4V, which had almost equal MIP and MBP of 3 - 4 %, the MBPs for all tested strains ranged from 3 to 30%. The inhibitory effect (MIP) against *E. coli* strains was more or less the same (6-7%) with the lethal concentration of MBP increasing from around twofold for 6C5V strain to fivefold for 5S8VR**.** Like *E. coli* 5S8VR, all tested CFSs had an antagonistic effect against *K. pneumonia*; it was inhibited and killed at 6 % and 30 %, respectively. Although *S. marcescens* 2S7N inhibited at the lowest concentration (2%), it required 10 times the MIP to kill it. *Ps. mohnii* 4L2V required a comparatively higher killing dose than *Ps. monteilii* 2Sa4V.

**Effect of catalase, pH neutralization, and heat treatment on the antibacterial activity of lactobacilli cell-free supernatants**

An entire loss of the antibacterial activity of the CFSs was observed when the CFSs'pH rose from 3.7 to 7.0. However, all catalase or heating-treated CFSs sustained most of their antagonistic activity as evidenced by the size of the growth-inhibition zones; such impact was *L. paracasei* strain and MDR/XDR target organism dependent (Table 4).

**Mode of action of *Lacticaseibacillus* CFSs antagonistic activities against MDR/XDR *E. coli* 6C5V and *Ps. mohnii* 4L2V strains**

TEM analysis examined the mode of action of the CFSs of *L. paracasei* strains (2S2, 4S2, 1R1, and 2R2) at 1.5 X MIP against MDR/XDR *E. coli* and *Ps. mohnii* strains. The cells' internal homogeneity and the integrity of their walls and membranes were compared to those of untreated control cells (Fig. 6 a and f). TEM images revealed normal morphology for untreated control, *E. coli*, and *Ps. mohnii* cells in terms of intact cell walls and membranes and consistent intracellular content. In contrast, the morphology of CFSs-treated MDR/XDR strains (Fig. 6 b-e and g-j) was strongly deformed. In addition to the inhomogeneity of intracellular material, there is a noticeable breakdown in both cell walls and membranes.

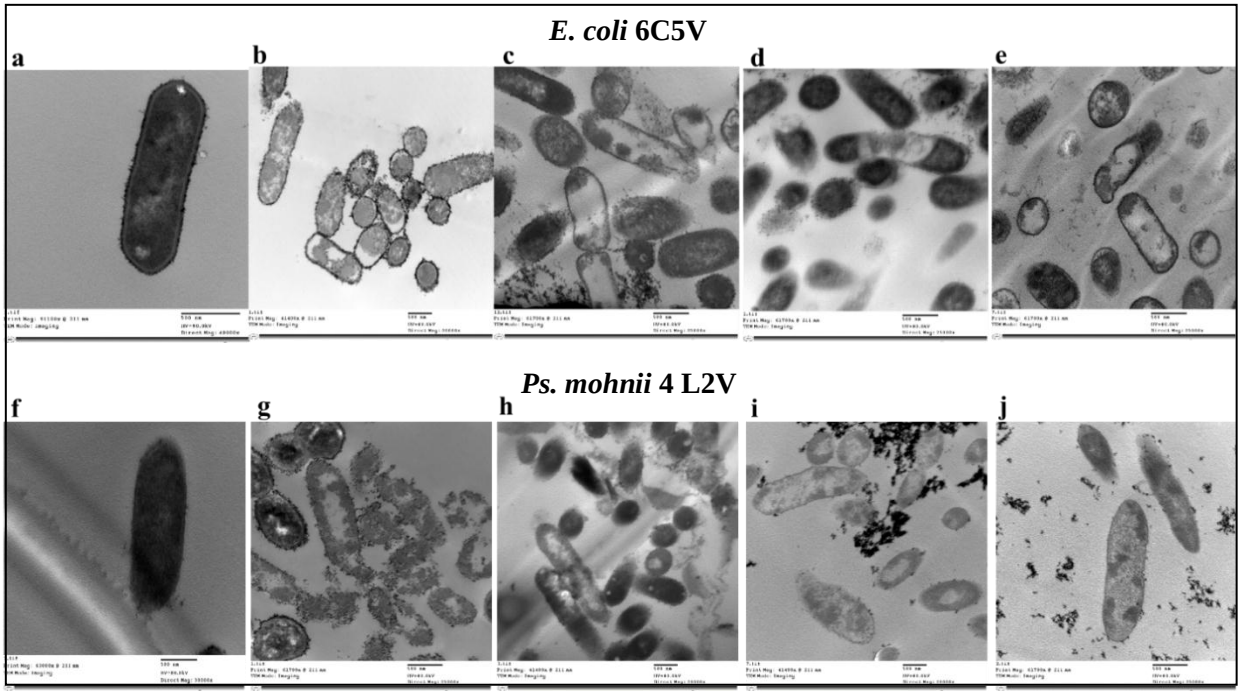



**Fig. 6.** TEM images of untreated (control) and treated MDR/XDR *E. coli* 6C5V and *Ps. mohnii* 4L2V strains with CFSs of *L. paracasei* strains (2S2, 4S2, 1R1, and 2R2) at 1.5 X MIP. a-f, untreated control; b-g, treated with 2S2-CFS; c-h, treated with 4S2-CFS; d-I, treated with 1R1-CFS; e-j, treated with 2R1-CFS

**Table 3** Antagonistic activities of cell-free-supernatants of *Lacticaseibacillus paracasei s*trains expressed as minimum inhibitory (MIP), and minimum bactericidal percentages (MBP) against selected multi-drug-resistant/extensively drug-resistant (MDR/XDR) strains of *Enterobacteriaceae* and *Pseudomonas* species.

| MDR/XDR strains | *L. paracasei* 2S2MRS | | | *L. paracasei* 4S2MRS | | | *L. paracasei* 1R1MRS | | | *L. paracasei* 2R1MRS | | | Antibiotics to which strains showed resistance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIP % | MBP % | MBP/MIP ratio | MIP % | MBP % | MBP/MIP ratio | MIP % | MBP % | MBP/MIP ratio | MIP % | MBP % | MBP/MIP ratio | |
| ***Enterobacter cloacae* 4C4N** | 7.00 | 10.00 | **1.43** | 6.00 | 15.00 | **2.50** | 6.00 | 15.00 | **2.50** | 6.00 | 15.00 | **2.50** | PIN, AMP, CTX, TET, and ERY. |
| ***Enterobacter hormaechei* 1S10V** | 6.00 | 10.00 | **1.67** | 6.00 | 10.00 | **1.67** | 6.00 | 10.00 | **1.67** | 6.00 | 10.00 | **1.67** | PIN, AMP, AMC, TET, NEO, ERY, and CHL. |
| ***Escherichia coli* 6C5 V** | 7.00 | 15.00 | **2.14** | 7.00 | 15.00 | **2.14** | 7.00 | 15.00 | **2.14** | 7.00 | 15.00 | **2.14** | PEN, AMP, CTX, MEM, AMC, TET, NEO, ERY, and CHL. |
| ***Escherichia coli* 5S8VR** | 6.00 | 30.00 | **5.00** | 6.00 | 30.00 | **5.00** | 6.00 | 30.00 | **5.00** | 6.00 | 30.00 | **5.00** | PEN, AMP, CTX, AMC, TET, NEO, ERY, CHL, and NAL. |
| ***Klebsiella pneumoniae* 3S10V** | 6.00 | 30.00 | **5.00** | 6.00 | 30.00 | **5.00** | 6.00 | 30.00 | **5.00** | 6.00 | 30.00 | **5.00** | PEN, AMP, CTX, TET, NEO, ERY, and CHL |
| ***Serratia marcescens* 2S7N** | 2.00 | 20.00 | **10.00** | 2.00 | 20.00 | **10.00** | 2.00 | 20.00 | **10.00** | 2.00 | 20.00 | **10.00** | PEN, AMP, CTX, PMB, AMC, TET, NEO, ERY, CHL, and NAL. |
| ***Pseudomonas monteilii* 2Sa4V** | 3.00 | 4.00 | **1.33** | 3.00 | 3.00 | **1.00** | 3.00 | 3.00 | **1.00** | 3.00 | 3.00 | **1.00** | PIN, AMP, CTX, MEM, ERY, CHL, and NAL. |
| ***Pseudomonas mohnii* 4L2V** | 2.00 | 6.00 | **3.00** | 2.00 | 6.00 | **3.00** | 2.00 | 8.00 | **4.00** | 2.00 | 5.0 | **2.50** | PEN, AMP, CTX, PMB, NEO, ERY, CHL, and NAL. |

SMRS, fermented sausage isolated on MRS medium; RMRS, fermented milk (Rayeb) isolated on MRS; PEN, penicillin; CTX, cefotaxime; MER, meropenem, AMC, augmentin (amoxicillin, 20 µg/clavulanic acid, 10 µg; PMB, polymyxin B; TET, tetracycline; NEO, neomycin; ERY, erythromycin; CHL, chloramphenicol and NAL, nalidixic acid.

**Table 4.** Antagonistic activities of cell-free-supernatants of *Lacticaseibacillus paracasei* strains as affected by pH neutralization, catalase, and different heat temperatures.

| ***L. casei* strains** | **Control** | **Neutral pH** | **catalase** | **Heat treatments** | | | |
|---|---|---|---|---|---|---|---|
| | | | | **80 °C/10 min** | **80 °C/30 min** | **100 °C/20 min** | **121 °C/15 min** |
| | **Inhibition zone (mm)** | | | | | | |
| | ***Enterobacter cloacae* 4C4N** | | | | | | |
| **2S2** | 14.33 | 0.0 | 14.33 | 13.67 | 15.00 | 15.67 | 16.67 |
| **4S2** | 13.67 | 0.0 | 13.00 | 13.33 | 15.33 | 14.00 | 15.33 |
| **1R1** | 13.67 | 0.0 | 12.33 | 14.67 | 16.33 | 17.33 | 15.00 |
| **2R1** | 14.33 | 0.0 | 12.00 | 13.33 | 14.33 | 16.00 | 15.00 |
| | ***Enterobacter. hormaechei* 1S10V** | | | | | | |
| **2S2** | 14.33 | 0.0 | 13.67 | 11.67 | 14.33 | 15.00 | 17.00 |
| **4S2** | 13.67 | 0.0 | 13.67 | 13.67 | 15.00 | 15.67 | 16.33 |
| **1R1** | 13.00 | 0.0 | 12.33 | 13.00 | 16.67 | 15.33 | 16.67 |
| **2R1** | 14.33 | 0.0 | 12.00 | 13.00 | 17.33 | 18.33 | 17.00 |
| | ***Escherichia coli* 6C5 V** | | | | | | |
| **2S2** | 16.33 | 0.0 | 15.67 | 14.67 | 14.67 | 15.00 | 16.67 |
| **4S2** | 16.33 | 0.0 | 13.67 | 13.33 | 14.00 | 15.00 | 14.00 |
| **1R1** | 12.67 | 0.0 | 12.67 | 12.33 | 13.67 | 16.67 | 14.00 |
| **2R1** | 18.00 | 0.0 | 12.33 | 12.67 | 13.67 | 15.00 | 13.33 |
| | ***Escherichia coli* 5S8VR** | | | | | | |
| **2S2** | 13.00 | 0.0 | 13.33 | 13.00 | 17.67 | 16.67 | 18.67 |
| **4S2** | 14.67 | 0.0 | 12.67 | 12.67 | 15.67 | 16.67 | 15.33 |
| **1R1** | 12.67 | 0.0 | 12.67 | 12.67 | 15.00 | 17.00 | 17.00 |
| **2R1** | 12.33 | 0.0 | 12.00 | 12.33 | 16.00 | 16.33 | 15.33 |
| | ***Klebsiella pneumoniae* 3S10V** | | | | | | |
| **2S2** | 14.67 | 0.0 | 12.33 | 14.33 | 16.33 | 16.00 | 15.67 |
| **4S2** | 12.33 | 0.0 | 12.67 | 12.67 | 16.67 | 16.67 | 15.00 |
| **1R1** | 13.00 | 0.0 | 12.00 | 12.00 | 16.33 | 19.33 | 16.67 |
| **2R1** | 12.33 | 0.0 | 12.00 | 12.67 | 15.00 | 17.33 | 16.67 |
| | ***Serratia marcescens* 2S7N A** | | | | | | |
| **2S2** | 24.67 | 0.0 | 19.67 | 24.33 | 15.67 | 17.67 | 15.67 |
| **4S2** | 20.33 | 0.0 | 20.33 | 18.67 | 15.00 | 15.00 | 14.33 |
| **1R1** | 22.00 | 0.0 | 18.00 | 21.00 | 17.00 | 16.33 | 15.67 |
| **2R1** | 21.67 | 0.0 | 18.33 | 19.33 | 13.33 | 18.00 | 15.33 |
| | ***Pseudomonas monteilii* 2Sa4V B** | | | | | | |
| **2S2** | 22.33 | 0.0 | 17.67 | 22.33 | 16.33 | 17.33 | 18.67 |
| **4S2** | 23.00 | 0.0 | 19.00 | 20.33 | 15.00 | 17.33 | 17.00 |
| **1R1** | 20.33 | 0.0 | 15.33 | 19.00 | 15.67 | 15.67 | 17.00 |
| **2R1** | 19.67 | 0.0 | 18.67 | 21.00 | 16.33 | 18.67 | 15.33 |
| | ***Pseudomonas mohnii* 4L2V** | | | | | | |
| **2S2** | 19.67 | 0 | 17.00 | 15.00 | 14.00 | 15.33 | 16.67 |
| **4S2** | 19.33 | 0 | 17.67 | 18.67 | 14.00 | 16.33 | 14.67 |
| **1R1** | 19.00 | 0 | 16.67 | 18.67 | 13.67 | 16.33 | 14.00 |
| **2R1** | 18.33 | 0 | 18.00 | 19.33 | 15.00 | 14.00 | 14.67 |
| **LSD value at 0.05 between:** | | | | | | | |
| **MDR/XDR strains** | | | **0.28** | **0.18** | | | |
| ***Lacticaseibacillus paracasei* strains** | | | **0.20** | **0.13** | | | |
| **Interaction** | | | **0.80** | **0.80** | | | |

## Discussion

*Enterobacteriaceae*, and *Pseudomonas* among other Gram-negative bacteria, are at the top of the list of MDR/XDR bacteria that pose the greatest intimidation to public health. Such intimidations are exacerbated by the increasing prevalence of these bacteria that resist the third-generation cephalosporins and carbapenems, increasing the likelihood of extended-spectrum beta-lactamases (ESBLs) producing strains among them. The situation becomes even more serious if the MDR/XDR strains resist colistin, which is considered the last resort antibiotic for treating infections. The menace of these MDR/XDR bacteria resides in the presence of pathogenic species of *Enterobacteriaceae* that cause community infections through contaminated food and opportunistic ones that cause hospital-acquired infections. Such bacteria threaten both individuals and their caregivers regarding difficulty and failure in treatment, medical expenditure, and subsequently heightened mortality rates [4, 5, 12, 43]. Overall, the jeopardy of MDR/XDR bacteria heightens from consumption of contaminated food along with the misapplication of antibiotics and also from healthy people whose gut become a reservoir for these bacteria; all of these help spread these bacteria and their resistant genes.

Aside from antibiotic resistance, the bacterial loads in terms of total aerobic count (TAC) and *Enterobacteriaceae* can all be useful in furnishing clues about the general quality of heat-processed RTE foods and the cogency of sanitation. TAC cannot be applied to indicate the microbiological quality of vegetable salad due to the expected high load of the natural bacteria associated with fresh vegetables. Besides, TAC could not be used as *Enterobacteriaceae* to predict the safety of RTE-food products [44, 45]. Based on microbiological quality criteria of RTE foods, the present study revealed that 73.33 % of luncheon meat, 60 % of both cheese and fermented sausage *samples with TAC results lying between* $10^5$ - $<10^7$ *CFU* $g^{-1}$ *were marginally acceptable quality;* 26.67% and 40% of luncheon meat and cheese samples were of unacceptable quality, with TAC exceeding $10^7$ and sometimes reached $10^9$ and $10^8$ *CFU* $g^{-1}$, respectively. Only 2 sausage samples met the microbiological criteria and the mean colony counts recorded $<10^5$ *CFU* $g^{-1}$. Concerning *Enterobacteriaceae*, only one luncheon sample was satisfactory with mean counts $< 10^2$; however, all sausage (100%), 36.36% of luncheon meat samples with counts lying between $>10^3$ - $<10^4$ *CFU* $g^{-1}$ were classified as marginally acceptable quality. All cheese samples and only one sample of luncheon meat were outside microbiological criteria, the *Enterobacteriaceae* counts exceeded $10^4$ *CFU* $g^{-1}$. *Enterobacteriaceae* is a good indicator for detecting digressions in compliance with good hygiene and contamination events during the processing, handling, and retailing of RTE foods. Thus, the greater risks of the potential presence of pathogenic species can be predicted. However, their detection in ready-to-eat vegetable salad may not indicate any processing failure due to their common presence in the environment besides human and animal guts.

The results of this investigation indicate that MDR bacteria are quite prevalent; from 12.77% of the examined RTE food samples, $> 10^5$ up to $10^7$ CFU $g^{-1}$ MDR bacterial populations were found. Moreover, MDR *Enterobacteriaceae* counts were found in cheese and salad at $>10^6$ and $>10^4$ CFU $g^{-1}$, respectively.

The MALDI-TOF MS method is built on a specific proteomic fingerprint that reflects microbial gene expression and metabolic products. It was proved to be a quick and reliable technique for identifying 74 of 75 (98.67%) MDR/XDR isolates to the genus level. Matching to 16S rRNA sequence analysis, the spectrometric method successfully identified all genetic variety strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter cloacae*, and some strains of *Enterobacter hormaechei* which represent 90.57 % (48 of 53 isolates) of *Enterobacteriaceae*. However, it was not in full congruence in identifying some strains of *Enterobacter* and *Pseudomonas* at the species level. Such results might be attributed to their complex taxonomy and regular evolution; therefore, they are still being revised due to genetic variation [46-48]. Although *Kluyvera cryocrescens* belongs to *Enterobacteriaceae*, it was not proteomically identified in the current study by MALDI-TOFMS. A compatible identification of this type of bacteria was obtained using 16S rRNA sequencing and the VITEK® 2 SYSTEM.

Several studies have found inconsistencies in freely available databases where the genomes of *Pseudomonas* species are either incorrectly classified at the species level or incorrectly recognized (*Pseudomonas* sp.). *Ps. putida* was discovered to be one of the lineage groups belonging to *Ps. fluorescens*. As a result, the misclassification of many strains in the *P. putida* group as *P. putida* occurs frequently [47 -50]. Based on the combination of MALDI-TOF MS and 16S rRNA gene sequencing, 45.45% of the *Pseudomonas* strains in the current study were identified as *Ps. monteilii* and *Ps. plecoglossicida*, which were allocated to the *Ps. putida* group. The remaining strains, however, were re-identified by

16 S rRNA gene sequencing to be *Pseudomonas* sp. (22.73%) and *Ps. mohnii* (31.82%). *Ps. mohnii* is included within the *Ps. jessenii* sub-group a cupboard of the phylogenetic *Ps. fluorescens*-related species [47 -51]. In general, the spectrum method's inability to accurately identify species may be due to the incompleteness of the proteome database, which forms its basis. The most notable technique for differentiating between species that ultimately belong to the same genus is still sequence analysis [46, 48].

*Pseudomonas* species are widely distributed in different environments because of their extremely versatile metabolism and capacity to adapt to diverse habitats with variable nutrients. They can outcompete other microbial groups in fresh foods causing aerobic deterioration at low temperatures by converting glucose to gluconate, which they can principally assimilate [49, 52, 53]. Lately, Kolbeck *et al*. [54] reported that *Pseudomonas* spp. can grow anaerobically in vacuum-packaged meat by converting arginine to ornithine *via* the arginine deiminase pathway, resulting in deterioration of these items. Remarkably, more recent research revealed the capacity of *Pseudomonas* to survive and endure heat processing of vacuum-sealed seasoned meat emulsion up to 71.1 $^{o}$C [55]. Their findings construe the results of the present study regarding the dominance of MDR/XDR *Pseudomonas* species in luncheon meats and fermented sausages; more than forty percent of ESBL producers were among these *Pseudomonas*.

Many *Enterobacteriaceae* and a few other Gram-negative organisms have chromosomes that encode AmpC B-lactamases. Likewise, transmissible plasmids could disperse genes for such enzymes. AmpC enzymes are therapeutically important cephalosporinases that mediate resistance to cephalosporins, and B-lactamase inhibitor/B-lactam combos. Plasmids harboring AmpC β-lactamases often contain resistance genes for aminoglycosides, chloramphenicol, quinolones, sulfonamide, tetracycline, and trimethoprim, as well as other β-lactamases [56 -58]. On the other hand, ESBL-producing genes are typically located on plasmids which are transferred by transposons or insertion sequences to facilitate their dissemination. ESBL-producing bacteria frequently have common resistance to aminoglycosides, tetracycline, and quinolones, but this tendency was not always genetically verified [59, 60]. Many mechanisms of bacterial resistance to colistin/polymyxin have been described; however, the most dangerous mechanism is plasmid-mediated horizontally transferred resistance (mcr1). Enterobacterales, *Pseudomonas*, and species were found to have mcr genes [10].

The current study reveals a significant prevalence of MDR/XDR, with all 75 *Enterobacteriaceae* and *Pseudomonas* strains categorized under MAR indices up to 0.91. This implies a likelihood of contamination from high-risk sources and an excessive and inappropriate use of antibiotics. Once salad and other RTE foods are consumed along with these bacterial species, the dissemination of such resistance genes and ESBL genes may transfer into the human gut, increasing the risk, particularly for the elderly, immunocompromised people, and those recuperating from illness, or indirectly from healthy people who become reservoirs for these bacteria that colonize their intestinal tract [20, 24, 59]. Therefore, RTE foods require proper procedures and a regular surveillance strategy to avoid such bacteria.

Consequently, knowing the prevalence of MDR/XDR bacteria in ready-to-eat food is imperative to determining how to cap their spread and control their growth and presence. Therefore, in the existing scenario, the principal objective of the current study was to search for and select lactic acid bacterial strains that have the potential to aid in overcoming multi-drug resistance (MDR/XDR). Several studies focused on using such bacteria as an alternative safe strategy to combat antibiotic-resistant bacteria; whether as whole cultures (probiotics) or as cell-free supernatants (CFSs) that contain the secreted bioactive exo metabolites [61]. Considering that, antimicrobial activity remains one of the primary criteria for choosing active strains, four lactic acid bacterial strains (2S2, 4S2; 1R1, and 2R1) were currently identified as *Lacticaseibacillus paracasei*; Their CFSs strongly inhibited all the recuperated MDR/XDR *Enterobacteriaceae* and *Pseudomonas* species from RTE foods. Lactic bacteria are known to inhibit/kill microorganisms by secreting exogenous metabolites such as organic acids, hydrogen peroxide, and ribosomal peptides, making them an alternate and safe control approach [62]. The inhibitory activity of exo metabolites generated by such strains is reflected by the diameter of the inhibition zones, bacteriostatic (MIP), and bactericidal activities (MBP) [33, 39, 63 - 66].

The antibacterial effect of *Lacticaseibacillus'CFSs* was completely abrogated when the pH was raised to 7.0, which is consistent with results previously obtained [33, 67, 68]. The steadiness of antibacterial activity at various pH levels was lactic acid bacterial strain-dependent; whole activity loss was obtained over a wide pH range of 5.0 to 11.0 [65, 66, 69 - 71]. The acidic environment seemed to be not solely responsible for the antibacterial effect; however, not all CFSs of LAB isolates showed antagonistic effects against the tested MDR/XDR bacteria, even though their growth dropped pH to

less than 4.0 in > 82% of cases (Tables S1-11). The dearth of inhibitory effect may be attributed to the loss of certain antimicrobial compounds' ability to adhere to and then detach from the producing cells [72] or the resistance of indicator microorganisms to low pH. Nonetheless, a preliminary experiment revealed that decreasing the pH of MRS broth with lactic acid had no antibacterial impact on the investigated MDR/XDR strains (data not shown). *Enterobacteriaceae* have been manifested to develop intricate mechanisms for tolerating and surviving low pH and organic acids [73, 74].

Concurrently, *Lacticaseibacillus'*CFSs upheld their antagonistic capacity after treatment with catalase and after heating even at high temperatures of up to 121 for 15 minutes at low pH. This indicates that most inhibitory components generated by *Lacticaseibacillus* strains are thermostable; such stability might be attributed to the low molecular weight and secondary structure [65, 70, 75, 76]. On the whole, the inhibitory activity of lactobacilli strains appears to be an integrated action dependent on all extracellular metabolites, including acid(s); acidity affects the cell surface charges of indicator organisms, making it easier to transport extracellular compounds found in CFSs through cell walls, henceforth enhancing bactericidal activity [68, 77, 79].

The entire lysis of treated MDR/XDR cells, as evidenced by TEM images, indicates the bactericidal capability of *Lacticaseibacillus* strains. The lethal effect could be attributable to the positively charged peptide(s) having a strong attraction to negatively charged membranes, triggering dysmorphology, intracellular inhomogeneity, and disintegration of the cell membrane via created pores, followed by cell lysis [80, 83, 84].

## Conclusion

*Lacticaseibacillus paracasei* 2S2, 4S2, 1R1, and 2R1 seemed a promising choice because they have the sturdiest antagonistic activities against all MDR/XDR *Enterobacteriaceae* and *Pseudomonas* species in foods. These potent strains should be investigated to see if they hurt other lactic acid bacteria strains used as starters. Furthermore, ongoing investigations that will confirm the safety of the strains and their desired features as live probiotics could be employed in the future as starters, in the formulation of nutraceuticals, and as bio-preservatives and food additives. Besides, additional research is needed to figure out their secreted metabolites.

## Conflicts of interest

The authors declare there are no conflicts of interest.

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## Authors' contribution

FMR, IED, and MAM conceptualized and designed the research. EEH conducted practical experiments; ASE performed DNA extraction, BOXPCR matching, and submission of the sequencing data to GenBank's database. FMR and EEH carried out statistical analysis and figured out data. FMR and ASE wrote the original draft. FMR interpreted the data, wrote, reviewed, edited, prepared the manuscript, and endorsed the version for publication. All authors read and approved the final manuscript.

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