ACTA MICROBIOLOGICA BULGARICA Volume 40 / 1 (2024)

Multidrug Resistance *Proteus* spp. in Meat and their Evaluation against Nano-Emulsion

Abitha Benson[1], Vinodkumar Selvaraj[2], Melvin Joe M[3*]

[1]*Department of Biotechnology, Karpaga Vinayaga College of Engineering, Chenai, India*
[2]*Department of Plant Pathology, SRM College of Agricultural Science, Faculty of Agriculture, SRM Institute of Science and Technology, India*
[3]*Department of Microbiology (Soil Science and Agricultural Chemistry), SRM College of Agricultural Science, Faculty of Agriculture, SRM Institute of Science and Technology, India*

Abstract

In the present evaluation, *Proteus* spp. isolated from the meat samples collected from the slaughterhouses were screened for their antibiotic susceptibility against Amikacin, Ampicillin, Ciprofloxacin, Colistin, and Gentamicin. Among the isolates, strain PBM32, showing a higher resistance toward most of the antibiotics was identified as *Proteus mirabilis* (NCBI Accession Number OR398497). As an alternative to the antibiotics, nanoemulsion was evaluated and it has significantly reduced (46% reduction) the motility of *P. mirabilis* PBM32 with respect to the control, and reduced the biofilm formation by 71.4%, 50%, and 55.5%, with respect to control, preservative and antibiotic treatments, respectively. Nanoemulsion treatment recorded a significant reduction of *P. mirabilis* PBM32 population in the pork samples and a reduction in mutton and beef samples when compared to other antimicrobials.

Keywords: *Proteus mirabilis,* antibiotics, drug resistance, nanoemulsion, survivability meat

Резюме

Щамове *Proteus* spp., изолирани от проби месо, събрани от кланиците, са изследвани за тяхната антибиотична чувствителност към амикацин, ампицилин, ципрофлоксацин, колистин и гентамицин. Сред изолатите, щам PBM32 показва по-висока резистентност към повечето антибиотици. Той е идентифициран като *Proteus mirabilis* (NCBI Accession Number OR398497). Като алтернатива на антибиотиците беше оценена наноемулсия, която значително намали (46% намаление) подвижността на *P. mirabilis* PBM32 спрямо контролата и намали образуването на биофилм със съответно 71.4%, 50% и 55.5% спрямо контролата, консерванта и антибиотичното третиране. Третирането с наноемулсия регистрира значително намаляване на популацията на *P. mirabilis* PBM32 в пробите от свинско месо и намаляване в пробите от овнешко и говеждо месо в сравнение с другите антимикробни средства.

Introduction

India processes over one million metric tonnes of meat annually, and Indian meat export accounts for the years 22–23 are about 2,022,390.94 metric tonnes of animal products (APEDA Report, 2023). However, about 3.5 billion kg of meat is wasted annually, causing significant economic and environmental impacts (Ajaykumar and Mandal, 2020).

Microorganisms are one of the major factors contributing to meat spoilage, and bacteria belonging to order *Enterobacteriales* are one of the most difficult-to-control contaminants in meat and meat products worldwide (Edris *et al.,* 2023). Among the order *Enterobacteriales*, and the family *Morganellaceae* (Adeolu *et al.,* 2016) *Proteus* species belong to gramme-negative, rod-shaped, saprophyte bacteria that have high swarming motility and urease activity and are of considerable interest in the meat industry (Armbruster *et al.,* 2018). Poor sanitation in traditional meat markets led to bacterial contamination of meat, including contamination by *Proteus mirabilis*, a human foodborne disease (Jamaluddin *et al.,* 2018). As an opportunistic pathogen, *P. mirabilis* is frequently linked to nosocomial infections, and *P. mirabilis* contamination in retail meat products may be a significant means of *P. mirabilis*-human infection transmission (Jiang *et al.,* 2017).

*Corresponding author: melvinjm@srmist.edu.in
Acta Microbiol. Bulg. 2024; 40(01). https://doi.org/10.59393/amb24400106

Areekit *et al.* (2019) reported that *P. mirabilis* can result in wound infections, CAUTI (catheter-associated urinary tract infections), and UTIs. According to Li *et al.* (2023), the prevalence of multi-drug-resistant (MDR) *P. mirabilis* restricts the use of antibiotics, which puts the public's health in danger. Integrative conjugative elements (ICEs) have been identified by Peng *et al.* (2023) as the key mobile elements connected to the spread of antibiotic resistance genes (ARGs) in *Proteus* sp. Antibiotic resistance is one of the major issues in the control of *P. mirabilis* strains, and about 48% of *P. mirabilis* strains exhibit antibiotic resistance, which in turn complicates infection by *P. mirabilis* (Girlich *et al.,* 2020). This scenario of antibiotic resistance in *P. mirabilis* strains urges the development of new antimicrobial agents for its control.

Nanoemulsions, which are emulsified by mixing oil, detergent, and water to a particle size of 100 to 800, have shown broad antimicrobial activity against diverse microorganisms coupled with no toxicity to animals (Hamouda *et al.,* 2001). According to a recent study by Fadel *et al.* (2023), the newly developed nano-gold/nano-Lavandula angustifolia effectively destroyed the microbial cells by penetrating the *P. mirabilis* biofilm. In this context, nanoemulsions possess nonspecific antibiotic resistance showing a broad spectrum of activity, thereby limiting the capability of antibiotic resistance generation in microorganisms (Hwang *et al.,* 2013), and this makes them an effective alternative to antibiotics.

In this study, antibiotic-resistant strains were isolated from meat samples collected from slaughterhouses. Strains showing multiple resistances were identified and tested against nanoemulsion for their efficacy in controlling drug-resistant *P. mirabilis*.

Materials and Methods

Bacterial isolation and identification

Meat samples that include pork, beef, and mutton were collected from the local slaughterhouses in Chengalpattu District, Tamil Nadu, India. Samples were collected in sterile containers maintained in ice boxes. For serial dilution, 25g of meat sample was diluted with 225 mL of PBS, homogenized in a shaker for 5 min, and incubated at 37°C for 24 h. This was followed by the subsequent transfer of one mL of the homogenate to 10 mL of BHI broth, incubated at 37°C for 24 h. This was followed by the subsequent transfer of 100 mL of the culture to xylose lysine deoxycholate agar, incubated at 37°C for 24 h. Suspected colonies of *P. mirabilis* were selected and confirmed based on molecular confirmation using primers UreR-F (GGTGAGATTTGTATTAATGG) and UreR-R (ATAATCTGGAAGATGACGAG) showing an amplicon size of 225 bp as described by Zhang *et al.* (2013). Four isolates from each group were evaluated for minimum inhibitory concentration (MIC) by the Kirby-Bauer disc diffusion method (Bauer *et al.,* 1966) and as per the protocols described by the manufacturer (Himedia, India). Discs used for this study had concentrations ranging from 256-0.001 µg/disc for Amikacin, 256-0.016 µg/disc for Ampicillin, 240-0.001 µg/disc for Ciprofloxacin, 240-0.001 µg/disc for Colistin, and 1024-0.064 µg/disc for Gentamicin. According to guidelines released by the Clinical and Laboratory Standards Institute (CLSI, 2008), the zone of inhibition was interpreted. For molecular identification, an isolate that showed resistance to a large number of antibiotics was chosen. Using bacterial 27F/1492R primers, a PCR reaction was carried out, amplified to a size comprising around 1,400 bp, and sequenced on an Applied Biosystems ABI 3730 sequencer using 518F/800R primers. Sequences were submitted to NCBI with Accession Number OR398497, and the phylogenetic tree was constructed using MEGA X software.

Swarming motility studies

The nanoemulsion (AUSN4) used in this study was synthesized as described previously by Hamouda *et al.* (1999) with the modifications and characterization described earlier in our previous study (Joe *et al.,* 2012). Sunflower oil makes up 80%, ethanol 2.5%, and polysorbate 80 makes 2.5%, and these components of the oil phase account for 85% (v/v) of the total emulsion. After the components of this oil phase were combined and heated to 86ºC for one hour, 15% water was added to make it an oil-in-water emulsion. The mixtures of oil-in-water emulsion were then centrifuged for five minutes at 15,000 rpm, and the result was passed through a high-pressure homogenizer operating at 2500 psi. Based on dynamic light scattering measurements, the resultant emulsion exhibits a mean droplet size of less than 50 nm. The determination of swarm motility as influenced by nanoemulsion treatment and other antimicrobials used for comparisons was evaluated as described by Kazemian *et al.* (2015). In brief, 50 µl of test components that include Ciprofloxacin 50 µg/ml, nanoemulsion 50 µl/ml, and sodium nitrate 50 µg/ml were mixed with 15 ml of MH agar medium and poured on sterile Petri dish-

es, and the plates were allowed to solidify. This was followed by point inoculation of a log-phase culture of *P. mirabilis* PBM32, incubated at 37°C for 72 h. The area of the colony was measured to determine the extent of swarm motility.

Biofilm inhibition assay

The effect of nanoemulsion treatment on the biofilm formation of *P. mirabilis* PBM32 was studied as described by Mirzaei *et al.* (2022). In brief, one hundred µl (OD_{620} = 0.1) of culture was added to 96-well microtiter plates and incubated for 72h at 37°C. Afterward, the media was discarded, followed by washing the biofilms with PBS (pH 7.2). For antimicrobial treatment, 50 µl of test components (Ciprofloxacin 50 µg/ml, nanoemulsion 50 µl/ml, and sodium nitrate 50 µg/ml) were added, and the contents were incubated separately for 18 h at 37°C. Wells containing biofilm were fixed with ethanol (90%), followed by 0.1% staining with crystal violet for 15 min, followed by washing in distilled water, solubilized in acetone and ethanol (1:1), and biofilm biomass was quantified by measuring the optical density at OD_{620} nm.

Survivability of P. mirabilis PBM32 in meat samples

The survivability of *P. mirabilis* PBM32 in meat samples as affected by different antimicrobials was determined as described by Ravishankar *et al.* (2009) with the required modifications. In brief, meat samples were cut into pieces (weighing 10 g) and dipped into boiling water (100°C) for 40 s to inactivate the native microorganisms. Samples were placed in petri dishes (lids open) in a UV chamber and dried for 30 minutes. Samples were inoculated with a log-phase culture (1 mL; 7 logs CFU/mL) of *P. mirabilis* PBM32. With a sterile pipette, the inoculum was dispersed on the surface as droplets and dried in an inoculation hood for 2h. Samples were wrapped in thin films containing either 50 µg/ml, nanoemulsion 50 µl/ml, or sodium nitrate 50 µg/ml.

Statistical analysis

Statistical analyses were done using one-way analysis of variance (ANOVA) with Tukey's honestly significant difference tests at a *p*-value of 0.05. A Minimum of six replications were maintained, and each experiment was repeated twice to ensure the data reproducibility.

Results and Discussion

The resistance of the strains to the studied antibiotics varied from 39–64 µg for Amikacin, 21–41 µmg for Ampicillin, 48–68 µg for Ciprofloxacin, 8–24 µg for Colistin, and 10–16 µg for Gentamicin (Fig. 1A). Among the 60 *Proteus* isolates screened for antibiotic sensitivity test, only one isolate (PBM32), which was later identified as *P. mirabilis* showed resistance against all the five tested antibiotics (Fig. 1B). Kim et al. (2005), based on their study on the antibiotic-resistant isolates belonging to order Enterobacteriales from retail meat products from Oklahoma reported *Proteus* sp. as the most resistant multidrug-resistant species.

Isolate PBM32 that exhibited resistance against all the antibiotics was identified as *Proteus mirabilis* based on 16S rDNA sequencing and the phylogenetic analysis of PBM32 with related *Proteus* strains is given in Fig. 2.

The sequence of this strain was submitted to NCBI with Accession Number OR398497. Analysis showed that this strain offered 99% similarity to *P. mirabilis* NCTC 11938. A recent study by Ma *et al.* (2022) reported higher contamination of retail meat and aquatic products with *P. mirabilis* harboring antimicrobial resistance genes of clinical significance. Nahar *et al.* (2014), based on their studies on the *P. mirabilis* isolates from chicken droppings, reported that 83% of *P. mirabilis* were MDR and found to be resistant to three or more antibiotics.

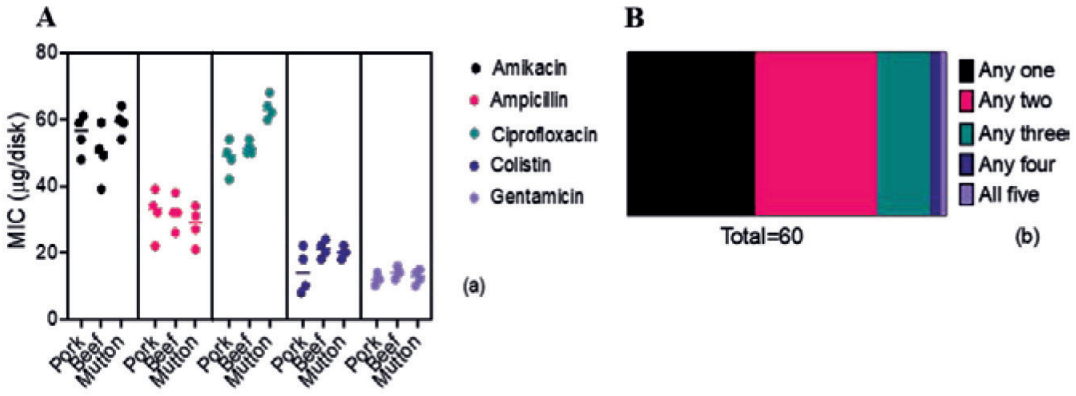

Fig. 1. MIC (**µg**/disk) of *Proteus* spp. from meat samples against selected antibiotics (A); resistance of antibiotics towards the evaluated 60 strains (B).
The mean value is represented as a bar.

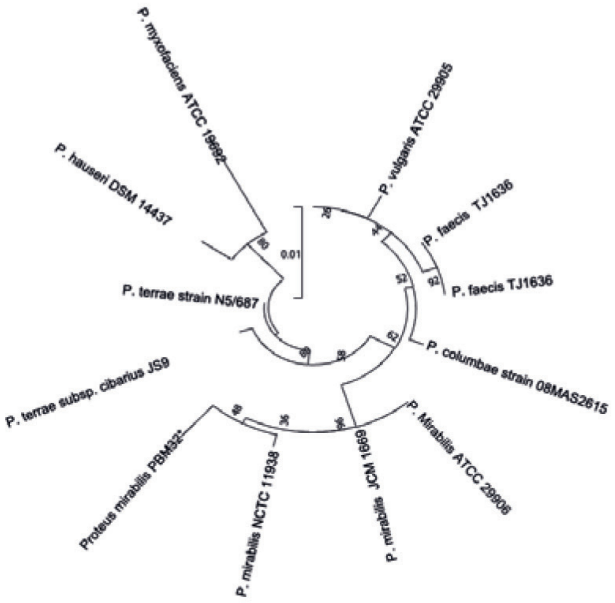

Fig. 2. Phylogenetic analysis of *Proteus mirabilis* PBM32 with related genera.
Phylogenetic tree constructed using MEGA-X, with neighbor-joining (NJ) with a bootstrap value of 1000

As an alternative to antibiotics, nanoemulsion was evaluated for its efficiency against multidrug-resistant *P. mirabilis* PBM32. Strains treated with different antimicrobials and evaluated for effect on swarming motility and biofilm were studied, and the results are presented in Fig. 3a, b. The influence of nanoemulsion on swarming motility and biofilm formation was studied because swarming motility allows migration of differentiated bacterial cells, and swarming shares many similarities with that of biofilm communities, notable for their antimicrobial resistance (Lai *et al.,* 2019). Nanoemulsion treatment significantly reduced (46% reduction) the motility of *P. mirabilis* PBM32 concerning control (Fig. 3A). However, no significant differences could be observed with concerning other antimicrobial treatments. Though not much research has been done on nanoemulsions against *P. mirabilis, a* recent report by Gomaa *et al.* (2022) reported that metformin-based nanoemulsion formulations at 1/10 MIC concentrations affected *P. aeruginosa* swarming motility compared to controls, and in particular, metformin-based nanoemulsions (MET-Nes) recorded maximum inhibition of swarming motility of 88.87–94.16%. Another study by Wang *et al.* (2018) reported that limonene-based nanoemulsions interfered with autoinducer2 (AI-2) quorum sensing in *E. coli* O157:H7, thereby reducing swimming and swarming motility. Interestingly, nanoemulsion treatment significantly reduced biofilm formation concerning other antimicrobial treatments and control (Fig. 3B).

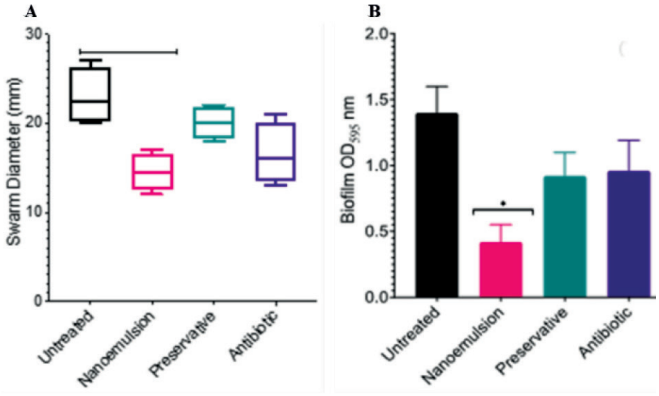

Fig. 3. Influence of nanoemulsion treatment on the motility (a) and biofilm formation (b) of Proteus mirabilis PBM32.
Values are a mean of six replications ± SD. Asterisks indicate a significant difference at *P*-value of 0.05.

Reduction in biofilm formation to the tune of 71.4%, 50%, and 55.5% with respect to control, preservative, and antibiotic treatments, respectively. These results on biofilm inhibition were similar to the earlier findings of Karthikeyan *et al.* (2010), who reported that nanoemulsion inhibited biofilm formation in *Streptococcus mutants* at the different studied concentrations of 100–250 µg, with the highest biofilm inhibition recorded at 250 µg. Another study by Jeong *et al.* (2021) reported that nanoemulsified cinnamon essential oil (CEO) inhibited multi-species oral biofilm maturation and microbial growth in oral biofilms, and aciduric bacteria in dental caries.

Nanoemulsion was compared for its efficiency in comparison with other antimicrobials for the reduction of *P. mirabilis* PBM32 population in PBS and on the meat surface, and the results are presented in Fig. 4. No significant differences could be observed among the studied antimicrobials in PBS, and on the meat surface when compared to other antimicrobial treatments. Nanoemulsion treatment recorded a 6.4 log reduction in the bacterial population, while antibiotic and preservative treatments recorded a 3.4 and 4.6 log reduction in the bacterial populations, respectively. González-González *et al.* (2021) reported that the nanoemulsified linalool treatment resulted in a reduction of pathogen levels with a corresponding increase in the washing time, resulting in 1.67- 1.83 log CFU/g reduction in *Salmonella* and *E. coli* populations, respectively. The latest study by Baghi *et al.* (2023) reported the antimicrobial efficiency of nanoemulsified linalool in inhibiting foodborne bacteria that include *E. coli* O157:H7, *S. Typhimurium,* and *S. senftenberg* on the growth of ground beef patties stored at 8°C for a period of ten days.

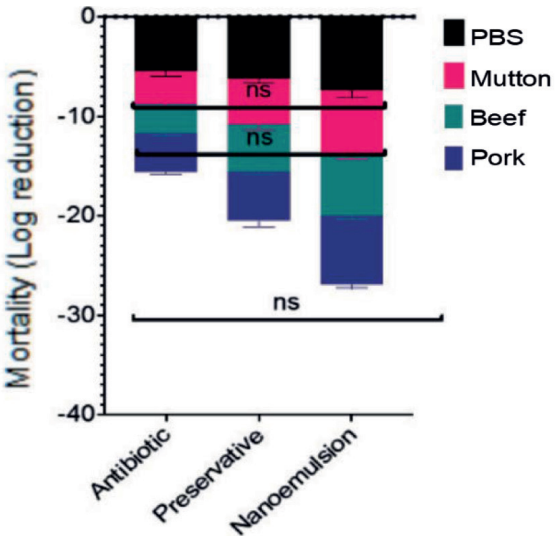

Fig. 4. Mortality of P. mirabilis strain JCM1669 in PBS and meat as influenced by nanoemulsion treatment.
NS- not significant.

Conclusions

The present study shows the prevalence of antibiotic-resistant *Proteus* spp. in meat, possibly due to unhygienic handling practices. Since most of the isolates showed resistance to more than one antibiotic, there has been an urge to exploit newer antimicrobials against these multidrug-resistant microbes in the food industry. In the present study, nanoemulsions have shown tremendous promise as newer-generation antimicrobials by inhibiting the swarming motility and biofilm formation of *P. mirabilis* under *in vitro* conditions and growth in meat under *in-situ* conditions. Nanoemulsions could be used to control antibiotic-resistant strains in the meat industry.

Acknowledgments

The authors thank the management of the institutions for the necessary support.

References

Adeolu, M., S. Alnajar, S. Naushad, R. S. Gupta (2016). Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. *Int. J. Syst. Evol. Microbiol.* **66**: 5575–5599. DOI:10.1099/ijsem.0.001485.

Ajaykumar, V. J., P. K. Mandal (2020). Chapter 18 - Modern concept and detection of spoilage in meat and meat products. In: Biswas, A. K., P. K. Mandal (Eds), Meat Quality Analysis, Academic Press, pp. 335-349. DOI:10.1016/B978-0-12-819233-7.00018-5.

APEDA (2023). Processed Meat-India Facts and Figures. https://agriexchange.apeda.gov.in/product_profile/prd_profile.aspx?categorycode=0404.

Areekit, S., N. Thongpramul, W. Yamprayoonswat, W. Jumpathong, S. Sittihan, S. Wanthongchareon, S. Ruangsuj, P Wachiralurpan, K. Chansiri, M. Yasawong (2019). Draft genome sequence of multidrug-resistant *Proteus mirabilis* CKTH01, isolated from raw chicken meat. *Microbiol. Resour. Announc.* **8**: e00861-19. DOI: 10.1128/MRA.00861-19.

Armbruster, C. E., H. Mobley, M. M. Pearson (2018). Pathogenesis of *Proteus mirabilis* infection. *EcoSal. Plus* **8**: 1-123. DOI:10.1128/ecosalplus.ESP-0009-2017.

Baghi, F., S. Ghnimi, E. Dumas, N. E. Chihib, A. Gharsallaoui (2023). Nanoemulsion-based multilayer films for ground beef preservation: antimicrobial activity and physicochemical properties. *Molecules* **28**: 4274. DOI:10.3390/molecules28114274.

Bauer, A. W., W. M. Kirby, J. C. Sherris, M. Turck (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**: 493-496. DOI:10.1093/ajcp/45.4_ts.493.

CLSI (Clinical and Laboratory Standards Institute) (2008). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, 2nd ed. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

Edris, S. N., A. Hamad, D. A. B. Awad, I. I. Sabeq (2023). Prevalence, antibiotic resistance patterns, and biofilm formation ability of *Enterobacterales* recovered from food of animal origin in Egypt. *Vet. World* **16**: 403-413. DOI:10.14202/vetworld.2023.403-413.

Fadel, B. A., B. H. Elwakil, E. E. Fawzy, M. M. Shaaban, Z. A. Olama (2023). Nanoemulsion of *Lavandula angustifolia* essential oil/gold nanoparticles: antibacterial effect against multidrug-resistant wound-causing bacteria. *Molecules* **28**: 6988. DOI:10.3390/molecules28196988.

Girlich, D., R. A. Bonnin, L. Dortet, T. Naas (2020). Genetics of acquired antibiotic resistance genes in *Proteus* spp. *Front. Microbiol.* **11**: 256. DOI:10.3389/fmicb.2020.00256.

Gomaa, S. E., G. H. Shaker, F. M. Mosallam, H. A. Abbas (2022). Knocking down *Pseudomonas aeruginosa* virulence by oral hypoglycemic metformin nano emulsion. *World J. Microbiol. Biotechnol.* **38**: 11. DOI:10.1007/s11274-022-03302-8.

González-González, C. R., O. Labo-Popoola, G. Delgado-Pando, K. Theodoridou, O. Doran, A. C. Stratakos (2021). The effect of cold atmospheric plasma and linalool nanoemulsions against *Escherichia coli* O157: H7 and *Salmonella* on ready-to-eat chicken meat. *LWT* **149**: 111898. DOI:10.1016/j.lwt.2021.111898.

Hamouda, T., A. Myc, B. Donovan, A. Y. Shih, J. D. Reuter, J. R. Baker (2001). A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. *Microbiol. Res.* **156**: 1–7. DOI: 10.1078/0944-5013-00069.

Hamouda, T., M. M. Hayes, Z. Cao, R. Tonda, K. Johnson, D. C. Wright, J. R. Baker (1999). A novel surfactant nanoemulsion with broad-spectrum sporicidal activity against *Bacillus* species. *J. Infect. Dis.* **180**: 1939-1949. DOI:10.1086/315124.

Hwang, Y. Y., K. Ramalingam, D. R. Bienek, V. Lee, T. You, R. Alvarez (2013). Antimicrobial activity of nanoemulsion in combination with cetylpyridinium chloride in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **57**: 3568-3575. DOI: 10.1128/

AAC.02109-12.

Jamaluddin, A. W., L. Muslimin, M. N. Djide (2018). Detection of *Proteus mirabilis* as foodborne disease bacteria in carcass of broiler chickens (*Gallus domesticus*). *J. Ind. Vet. Res.* **2**: 35-40. DOI:10.20956/jrvi.v2i1.4386.

Jeong, Y. J., H. E. Kim, S. J. Han, C. Jun-Seuon (2021). Antibacterial and antibiofilm activities of cinnamon essential oil nanoemulsion against multi-species oral biofilms. *Sci. Rep.* **11**: 5911. DOI: 10.1038/s41598-021-85375-3.

Jiang, X., T. Yu, L. Liu, Y. Li, K. Zhang, H. Wang, L. Shi (2017). Examination of quaternary ammonium compound resistance in *Proteus mirabilis* isolated from cooked meat products in China. *Front. Microbiol.* **8**: 2417. DOI: 10.3389/fmicb.2017.02417.

Joe, M. M., P. S. Chauhan, K. Bradeepa, C. Shagol, P. K. Sivakumar, T. Sa (2012). Influence of Sunflower oil based nanoemulsion (AUSN-4) on the shelf life and quality of Indo-Pacific king mackerel (*Scomberomorus guttatus*) steaks stored at 20°C. *Food Control* **23**: 564–570. DOI:10.1016/j.foodcont.2011.08.032.

Karthikeyan, R., B. T. Amaechi, H. R. Rawls, V. A. Lee (2010). Antimicrobial activity of nanoemulsion on cariogenic *Streptococcus mutans*. *Arch. Oral Biol.* **56**: 437-445. DOI: 10.1016/j.archoralbio.2010.10.022.

Kazemian, H., S. Ghafourian, H. Heidari, P. Amiri, J. K. Yamchi, A. Shavalipour, H. Houri, A. Maleki, N. Sadeghifard (2015). Antibacterial, anti-swarming and anti-biofilm formation activities of *Chamaemelum nobile* against *Pseudomonas aeruginosa*. *Rev. Soc. Bras. Med. Tro.* **48**: 432-436. DOI: 10.1590/0037-8682-0065-2015.

Kim, S. H., C. I. Wei, H. An (2005). Molecular characterization of multidrug-resistant *Proteus mirabilis* isolates from retail meat products. *J. Food Protect.* **68**: 1408-1413. DOI: 10.4315/0362-028x-68.7.1408.

Lai, S., J. Tremblay, E. Déziel (2009). Swarming motility: a multicellular behaviour conferring antimicrobial resistance. *Environ. Microbiol.* **11**: 126-136. DOI: 10.1111/j.1462-2920.2008.01747.x.

Li, Y., M. Yin, C. Fang, Y. Fu, X. Dai, W. Zeng, L. Zhang (2023). Genetic analysis of resistance and virulence characteristics of clinical multidrug-resistant *Proteus mirabilis* isolates. *Front. Cell. Infect. Microbiol.* **13**: 1229194. DOI: 10.3389/fcimb.2023.1229194.

Ma, W. Q., Y. Y. Han, L. Zhou, W. Q. Peng, L. Y. Mao, X. Yang, Q. Wang, T. J. Zhang, H. N. Wang, C. W. Lei (2022). Contamination of *Proteus mirabilis* harbouring various clinically important antimicrobial resistance genes in retail meat and aquatic products from food markets in China. *Front. Microbiol.* **13**: 1086800. DOI:10.3389/fmicb.2022.1086800.

Mirzaei, A., B. N. Esfahani, M. Ghanadian (2022). *Alhagi maurorum* extract modulates quorum sensing genes and biofilm formation in *Proteus mirabilis*. *Sci. Rep.* **12**: 13992. DOI: 10.1038/s41598-022-18362-x.

Nahar, A., M. Siddiquee, S. Nahar, K.S. Anwar, S. Islam (2014). Multidrug-resistant *Proteus mirabilis* isolated from chicken droppings in commercial poultry farms: bio-security concern and emerging public health threat in Bangladesh. *J. Biosaf. Health Educ*. **2**: 1-5. DOI: 10.4172/2332-0893.1000120.

Peng, K., Y. Li, Q. Wang, P. Yang, Z. Wang, R. Li (2023). Integrative conjugative elements mediate the high prevalence of tmexCD3-toprJ1b in *Proteus* spp. of animal source. *mSystems* **8**: e00429-23. DOI: 10.1128/msystems.00429-23 1.

Ravishankar, S., L. Zhu, C. W. Olsen, T. H. McHugh, M. Friedman (2009). Edible apple film wraps containing plant antimicrobials inactivate foodborne pathogens on meat and poultry products. *J. Food Sci.* **74**: M440-M445. DOI: 10.1111/j.1750-3841.2009.01320.x.

Wang, R., P. Vega, Y. Xu, Y. Chen, J. Irudayaraj (2018). Exploring the anti-quorum sensing activity of a d-limonene nanoemulsion for *Escherichia coli* O157:H7. *J. Biomed. Mater. Res. A.* **106**: 1979-1986. DOI: 10.1002/jbm.a.36404.

Zhang, Z., K. Niu, P. Yin, L. Liu (2013). Chen quick identification and quantification of *Proteus mirabilis* by polymerase chain reaction (PCR) assays. *Ann. Microbiol.* **63**: 683-689. DOI 10.1007/s13213-012-0520-x.