

# Antibiotic resistance in bacteria strains isolated from foods and correlated environments

*Pasqualina Laganà<sup>1</sup>, Santi Delia<sup>1</sup>, Angela Di Pietro<sup>2</sup>, Alessandro Costa<sup>2</sup>, Maria Anna Coniglio<sup>3</sup>*

<sup>1</sup>Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy.

<sup>2</sup>Biologist, University of Messina, Messina, Italy. <sup>3</sup>Department 'G.F. Ingrassia', University of Catania, Catania, Italy,

**Summary.** Antibiotic resistance is the natural consequence of an evolutionary adaptation of bacteria. The phenomenon is constantly increasing due to the widespread use of antibiotics, not only in human therapy, but also in zootechny and veterinary medicine with the consequent of a rapid selection of antibiotic-resistant strains. Food plays an important role in the development and spread of antibiotic resistance. Antibiotic use in food animals due to treatment, prevention or growth promotion, allows resistant bacteria and resistance genes to pass from food animals to humans through the food chain.

The antibiotic resistance of 76 strains from food and from the food sector environment was assessed through the execution of antibiograms carried out with the Kirby-Bauer method. The general results of the sensitivity tests performed on the strains indicated that Gram negative bacteria showed more than 50% of resistance to the 55% of the tested antibiotics (21 resistant strains out of 38 molecules used). Moreover, Gram positive bacteria showed a resistance greater than 50% towards 14% of antibiotics (12 resistant strains out of 32 molecules used). The Authors conclude by hoping for a greater awareness in the use of antibiotics both in the human and veterinary fields.

**Key words:** Antibiotic resistance, Food, Environment, Human health risk.

## Introduction

Antibiotic resistance is the natural consequence of an evolutionary adaptation of bacteria due to the exposition to antimicrobial agents. The phenomenon is constantly increasing due to the widespread use of antibiotics, not only in human therapy, but also in zootechny and veterinary medicine with the consequence of resistant strains rapid selection. Studies carried out on bacteria isolated in extreme environments, such as Arctic and Antarctic regions, have shown the presence of antibiotic resistance as well as the most disparate vectors have been identified (1-4). The dramatic consequence of the selection of antibiotic-resistance bacteria

is the loss of therapeutic efficacy of these molecules with serious health risks for both humans and animals. In fact, recent estimates based on data from EARS-Net (European Antimicrobial Resistance Surveillance Network) show that each year, more than 670000 infections occur in the EU/EEA due to bacteria resistant to antibiotics, and that approximately 33000 people die as a direct consequence of these infections. The related cost to the healthcare systems of EU/EEA countries is around EUR 1.1 billion (5).

The phenomenon is also a food safety problem: antibiotic use in food animals due to treatment, prevention or growth promotion, allows resistant bacteria and resistance genes to pass from food animals to hu-

mans through the food chain (6). The foods that we consume, especially if raw, can be contaminated by bacteria and therefore represent a potential transmission pathway of resistant strains (7).

Different authorities are responsible for Food Safety all over the world. In the USA the FDA (Food and Drug Administration), in China the CFDA (Chinese Food and Drug Administration), in Brasil the ANVISA (Agência Nacional de Vigilância Sanitária), for example, are responsible for protecting the public health ensuring the safety, security and efficacy of national food supplies. In Europe the EFSA (European Food Safety Authority) assesses the risks related to animal feed safety and EFSA and ECDC (European Centre for disease Control) supervise antibiotic resistance in animals and humans, using data reported by Member States. These two last agencies cooperate with EMA (European Medicines Agency) to analyze the relationship between use of antibiotics and resistance in both food producing animals and humans.

The modes of infections transmission are various and widely discussed in recent years by many authors, some classic others more whimsical, both in community and hospital settings, as *foods, air, pediatric incubators, dental units, hands* (8–10) Anyway, the most common food-borne diseases are caused by resistant strains of *Salmonella* (11), *Campylobacter* (12), and the foods mostly involved in the transmission of these bacteria are poultry and pig meat, cattle and eggs. Also vegetables can be contaminated by animal waste or water not suitable for human consumption (13, 14).

Finally, antibiotic resistance can be transmitted to humans by foods through various mechanisms:

- direct transmission (via animal foods carrying resistant bacteria that can colonize or infect man after ingestion).
- resistance transfer (through food contaminated by resistant bacteria during the preparation, handling and processing phases).
- ingestion of resistant bacteria (through fresh contaminated products, as in aquaculture and horticulture products).

Taking into consideration what has been already argued above, the purpose of this study was to evaluate the antibiotic resistance in bacteria isolated from food

and in the food sector to strengthen the idea that foods are very often involved in the spreading and circulation of antibiotic-resistant bacteria..

## Materials and Methods

### *Origin of Strains*

The bacteria used in this research were taken from the collection of the Microbiological Laboratory, of the Department of Biomedical Sciences and Images Morphological and Functional, Section of Medicine Preventive, University of Messina (Italy). Preliminary bacteria isolation was done using medium usually utilized for food and environmental microbiological qualitative analysis.

In total, 76 bacterial strains were used: 33 isolated from potable water, 23 isolated from foods (salad, mozzarella, meat, etc.), 15 from alimentary surfaces (work bench, tables, chopping board, etc.) and 5 strains from tools used in cooking (slicer, cutlery, saucepan, etc.).

The strains studied were both Gram negative (*Pseudomonas aeruginosa*, *P. luteola*, *Stenotrophomonas maltophilia*, *Klebsiella oxytoca*, *Escherichia coli*, *Citrobacter freundii*, *Proteus mirabilis*, *Enterobacter agglomerans*, *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Shewanella putrefaciens*, and Gram positive (*Staphylococcus aureus*, *S. sciuri*, *S. xylosus*, *S. conii*, *S. epidermidis*).

### *Isolation and phenotypic identification*

The isolates were identified to the species or genus level by API 20 NE, API 20 E and API Staph profiles (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions.

### *Antimicrobial susceptibility*

The bacterial isolates were tested for resistance or sensitivity to different antimicrobial agents using the standard disk diffusion method (Kirby Bauer test) (15). After revitalising the bacterial strains in Brain Heart Infusion (Oxoid), bacteria were grown for 48 h on plates of Tryptic Soy Agar (Oxoid), harvested and

then suspended in sterile water adjusted to 0.5 McFarland turbidity standard (bioMérieux), corresponding to  $1.5 \times 10^8$  CFU mL<sup>-1</sup>. The inoculum was streaked onto plates of Mueller-Hinton agar using a cotton swab; results were read after 24 h of incubation at 37°C.

Commercially available antibacterial disks, obtained from Oxoid were used to determine the resistance patterns of the isolates against 38 different antibacterial (disk/dose) for Gram negative bacteria and 36 for Gram positive, grouped into three specific classes (Cell wall antibiotics, Nucleic acid inhibitors and Protein synthesis inhibitors) according to their different mechanisms of action. The diameter of the zone of inhibition around each disk was measured with a precision caliper (Mitutoyo, Andover, UK). Each bacterial species was classified as Resistant (R), Intermediately resistant (I) or Sensitive (S) according to the breakpoints established by the EUCAST (2017). For cinoxacin and sisomicin molecules, the breakpoints established by Clinical Laboratory Standards Institute (CLSI, 2012) were used.

#### *Antibiotic molecules used*

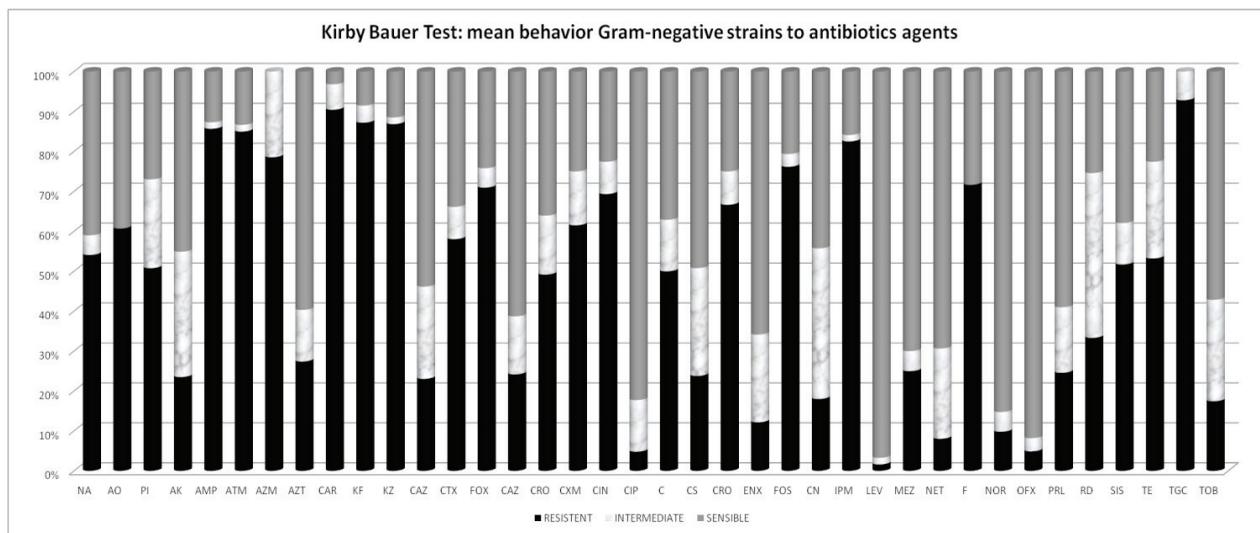
The antibiotic molecules used to carry out the antibiograms are the most used in the human field. Sometimes the same molecules were used for both Gram negative and Gram positive bacteria because we wanted to evaluate a possible broad spectrum antibiotic resistance pattern.

*For Gram negative bacteria:* Nalidixic Acid (NA); Oxolinic Acid (AO); Pipemidic Acid (PI); Amikacin (AK); Ampicillin (AMP); Aztreonam (ATM); Azithromycin (AZM); Amoxicillin and Clavulanic Acid (AZT); Carbenicillin (CAR); Cefalotin (KF); Cefazolin (KZ); Ceftazidime (CAZ); Cefotaxime (CTX); Cefoxitin (FOX); Ceftazidime (CAZ); Ceftriaxone (CRO); Cefuroxime (CXM); Cinoxacin (CIN); Ciprofloxacin (CIP); Chloramphenicol (C); Colistin Sulphate (CS); Ceftriaxone (CRO); Enoxacin (ENX); Fosfomycin (FOS); Gentamycin (CN); Imipenem (IPM); Levofloxacin (LEV); Mezlocillin (MEZ); Netilmicin (NET); Nitrofurantoin (F); Norfloxacin (NOR); Ofloxacin (OFX); Piperacillin (PRL); Rifampicin (RD); Sisomicin (SIS); Tetracycline (TE); Tigecycline (TGC); Tobramycin (TOB).

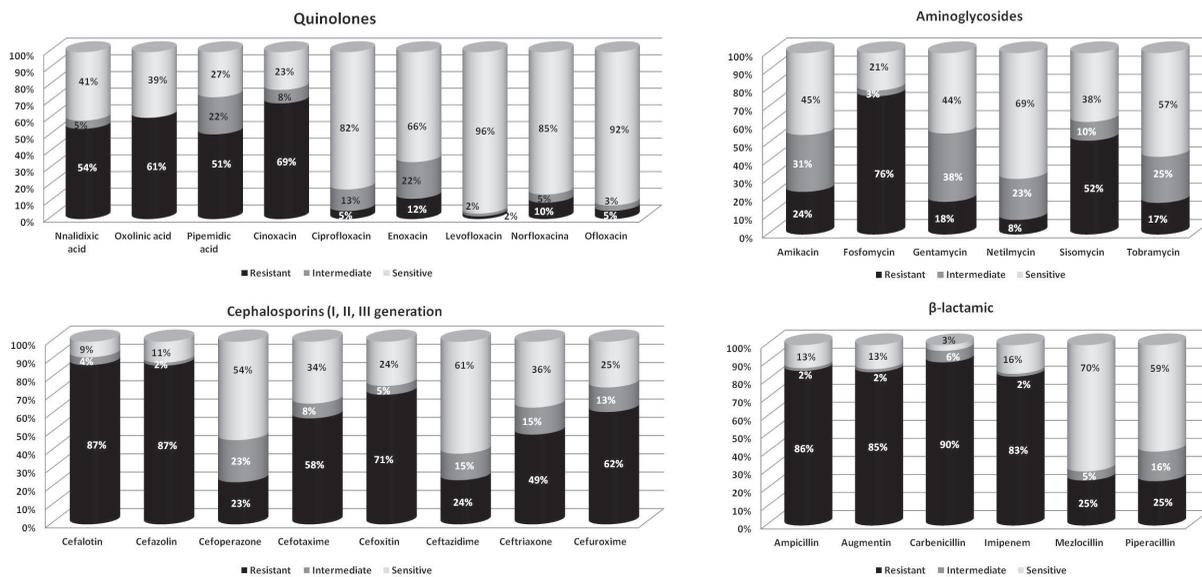
*For Gram positive bacteria:* Amikacin (AK); Amoxicillin (AMX); Ampicillin (AMP); Amoxicillin and Clavulanic Acid (AZT); Carbenicillin (CAR); Cephalexin (CL); Cefalotin (KF); Cefazolin (KZ); Cefotaxime (CTX); Ceftriaxone (CRO); Cefuroxime (CXM); Cinoxacin (CIN); Ciprofloxacin (CIP); Clindamycin (DA); Chloramphenicol (C); Sulphamethoxazole + trimethoprim (SXT); Doxycycline (DO); Enoxacin (ENX); Erythromycin (E); Fosfomycin (FOS); Gentamycin (CN); Imipenem (IPM); Levofloxacin (LEV); Josamycin (JOS); Mezlocillin (MEZ); Minocycline (MH); Nitrofurantoin (F); Norfloxacin (NOR); Ofloxacin (OFX); Oxacillin (OX); Penicillin (P); Piperacillin (PRL); Rifampicin (RD); Sisomicin (SIS); Tetracycline (TE); Vancomycin (VAN).

#### **Results**

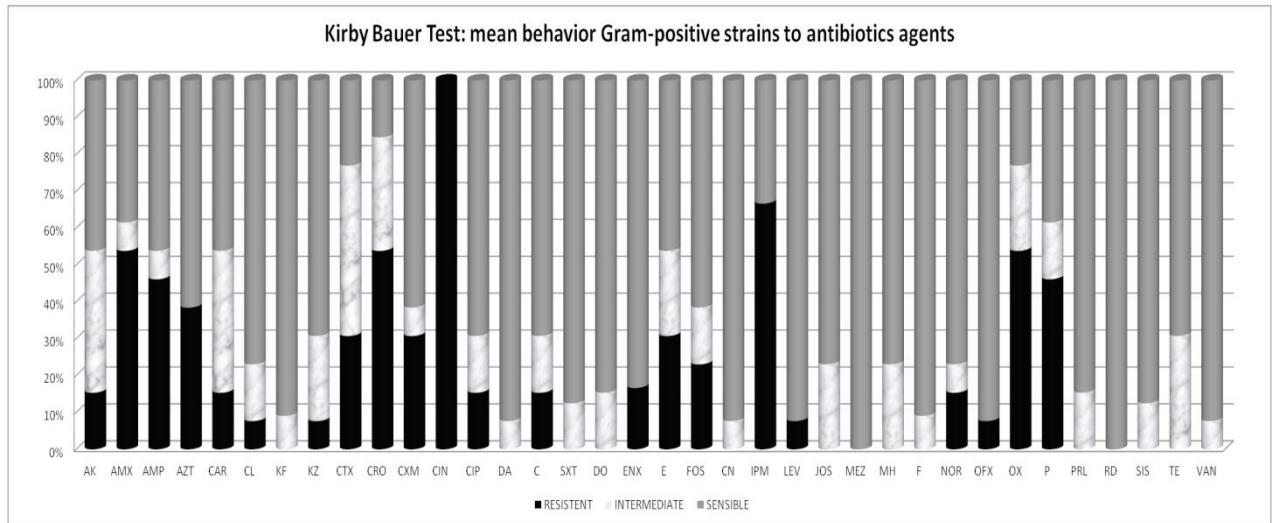
General results of susceptibility testing performed on the considered strains are reported in Fig. 1 (Gram negative) and Fig. 3 (Gram positive). Gram negative bacteria showed more than 50% of resistance to the 55% of the tested antibiotics (21 resistant strains out of 38 molecules used). Gram positive bacteria showed a resistance greater than 50% towards 14% of antibiotics (12 resistant strains out of 32 molecules used). Gram negative bacteria showed a greater resistance to antibiotics compared to the Gram positive ones. In particular, considering the different classes of antibiotics, resistance to  $\beta$ -lactamic was detected in a high percentage of Gram negative strains (from 83 to 90%), and less in Gram positive (from 15 to 67%). Taking into consideration cephalosporins, resistances were found in a relevant percentage of Gram negative bacteria (from 23 to 87%), less in Gram positive (from 8 to 54%). Resistance to quinolones was observed in a smaller number of isolates: from 5 to 69% of Gram negative, while the Gram positive strains showed a high sensitivity (only from 8 to 15% were resistant). A similar pattern was observed for the aminoglycosides group: from 8 to 76% of Gram negative showed resistance towards the molecule, while the Gram positive strains were almost totally sensitive to it (Fig. 2 and 4).



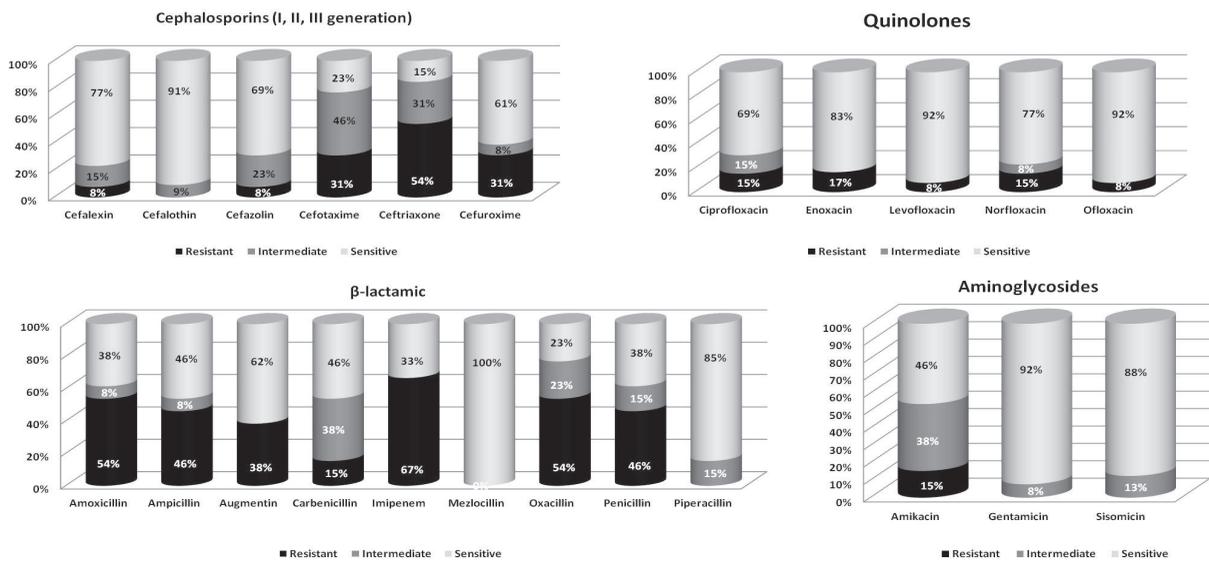
**Figure 1.** Antibiotic susceptibility profiles of the Gram-negative bacteria isolated. Percentages of resistant, intermediate or sensitive strains compared to the total of the isolates considered for the study, as obtained from Kirby-Bauer test performed against each assayed antibiotic molecule.



**Figure 2.** Antibiotic susceptibility profiles of isolated Gram-negative bacteria grouped by the main classes of molecules as obtained from the Kirby-Bauer test.



**Figure 3.** Antibiotic susceptibility profiles of the Gram-positive bacteria isolated. Percentages of resistant, intermediate or sensitive strains compared to the total of the isolates considered for the study, as obtained from Kirby-Bauer test performed against each assayed antibiotic molecule.



**Figure 4.** Antibiotic susceptibility profiles of isolated Gram-positive bacteria grouped by the main classes of molecules as obtained from the Kirby-Bauer test.

### Conclusion and Discussion

Many initiatives have been undertaken to monitor the knowledge of the population on Public Health topics that affect each of us. For example, in recent years, interesting questionnaires have been distributed aimed at detecting knowledge on

antibiotic resistance, both in health professionals and in the general population, often concurrently with World Antibiotic Awareness Week (16, 17), on vaccinations (18, 19) or on obesity (20). Knowledge is not always at high levels and this means that we still have a lot of work to do to understand how the spread of these phenomena can affect human

health and how important it is to undertake contrast actions.

Antibiotic resistance is a phenomenon that has been cause of concern at various levels for years. Excessive or incorrect use of antibiotics is considered to be the main cause of the development and spread of resistant microorganisms. The dramatic consequence is the loss of therapeutic efficacy with serious risks for public health, especially for humans but also for animals.

The burden of antibiotic-resistant bacterial strains is often faster than the development of new active molecules, thus becoming a real problem for health-care organizations, both for the clinical implications, such as increased mortality, duration of diseases, development of complications and the possibility of epidemics, and for the considerable economic expenditure linked to the delay of hospitalizations and the need to recur to much more expensive last generation drugs. Knowledge of the mechanisms of bacterial resistance and the development of new drugs with antimicrobial activity are essential factors to limit the damage and keep a possibility of treatment open.

Over the past few years much attention has been paid by the scientific community to the study of the resistance mechanisms of pathogenic bacteria (21-25). Despite this, in many cases these studies have provided little information on the origin and sources of antibiotic resistance. A broader view of this phenomenon ought to include the resistance genes present in the genomes of both pathogenic and non-pathogenic species. For this reason, the term 'resistome' has been used to describe the collection of all resistance genes present in all microbial genomes. The genes that code for resistance to various antibiotics are generally located in extra-chromosomal units (R-plasmids) which make it easy to transfer between the various microbial species, especially at the level of the intestinal microflora. (26). The effects on human health are significant, as the resistant bacteria selected following the use of antibiotics in animals can be transmitted to humans and in turn transfer the resistances to other bacteria that cause diseases. In 2018, Blau K. et al. (27). characterized the transferable resistome of bacteria isolated from mixed rocket and coriander salad purchased in German supermarkets. Researchers isolated tetracycline-resistant

*E. coli* from these vegetables and showed that almost all the isolates were resistant to at least one class of antibiotics, while two isolates were resistant to eight classes of antibiotics. The study showed the presence of plasmids in the studied strains carrying multiple and self-transmissible resistances, which could play a role in the spread of antimicrobial resistance. In the same year, MacFadden DR et al (28) found a correlation between the antibiotic resistance detected in strains of very common bacteria (*E. coli*, *K. pneumoniae* and *S. aureus*) and the local weather-climatic data. The results demonstrated a statistically significant link between the increase in the minimum temperature and the increase in antibiotic resistance. In particular, after taking into account factors that could lead to an increase in the phenomenon of the antibiotic resistance, such as an increase in population density or in antibiotic prescriptions, the researchers demonstrated that a difference of 10°C in the minimum temperatures between various areas of the USA leads to differences ranging from 2.2 to 4.2% in the number of infections sustained by antibiotic-resistant bacteria. As evidence of the fact that environmental factors have a significant relevance in the onset of antibiotic resistance, there are many scientific publications (29, 30), Diwan V et al. (31), for example demonstrated the importance of seasonal variation in water quality, antibiotic residue levels, antibiotic resistance genes and antibiotic resistance in *E. coli* strains isolated from the water and sediments of the Kshipra River in the Central India.

Moreover, environmental stress in general can play a role in determining some alterations of the membranes of both human and animal cells (e.g. erythrocytes), so they must be taken into consideration to prevent physiological damage. These damages can be caused not only by environmental stress but also by disorder caused by infections induced by pathogenic bacteria or viruses or by inflammations (32, 33). Taking into consideration the food sector, it is easy to hypothesize how the antibiotic resistance found in isolated bacterial strain, can potentially be transferred to the bacterial intestinal flora of hospitalized and non-hospitalized subjects, contributing significantly to the failure of the traditional anti-microbial therapies.

To contrast the phenomenon of antibiotic resistance, the use of various natural molecules has also been

proposed, such as honey, foods that contain polyphenols, citrus essential oils, etc. (34-36).

The 'Risk Communication' could also be important, making consumers more aware of their choices and more careful in handling and preparing food. (37-40). Finally, surveillance at the local level is essential to follow the trend of the phenomenon and to plan suitable interventions to control the spread and define the best therapeutic strategies. Surveillance should be carried out continuously and above all with reference to the local situation. In any case it is always useful to keep in mind some recommendations, often repeated but not as often implemented, such as i) choose the antibiotic molecule on the basis of the antibiograms carried out on the strain isolated from the subject with the infection; ii) pay attention to the doses and time of administration of the therapy; iii) avoid 'antibiotic cocktails'; iv) when possible prefer topical to systemic medications; v) avoid using antibiotics when it is not necessary; vi) implement the use of strict asepsis procedures, especially in the hospital environment, to limit the circulation of bacteria.

**“No potential conflict of interest relevant to this article was reported by the authors”.**

## References

- Laganà P, Votano L, Caruso G, et al. Bacterial isolates from the Arctic region (Pasvik River, Norway): assessment of biofilm production and antibiotic susceptibility profiles. *Environmental Science and Pollution Research* 2018; 25:1089–1102.
- Laganà P, Caruso G, Corsi I, et al. Do plastics serve as a possible vector for the spread of antibiotic resistance? First insights from bacteria associated to a polystyrene piece from King George Island (Antarctica). *Int J Hyg Environ Health*. 2019 Jan;222(1):89-100.
- Moore RE, Millarb BC, Moore JE. Antimicrobial resistance (AMR) and marine plastics: Can food packaging litter act as a dispersal mechanism for AMR in oceanic environments? *Marine Pollution Bulletin* 150 (2020) 1107022
- Caruso G. Microplastics as vectors of contaminants. *Marine Pollution Bulletin*. 2019; 146: 921-924.
- Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Surveillance of antimicrobial resistance in Europe 2018. Accessible to: <https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2018.pdf>
- Founou LL, Founou RC, Essack SY. Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. *Front Microbiol*. 2016; 7:1881.
- Lammie SL and Hughes JM. Antimicrobial Resistance, Food Safety, and One Health: The Need for Convergence. *Annual Review of Food Science and Technology*. 2016; 7:287-312
- Stilo A, Troiano G, Melcarne L, et al. Hand washing in operating room: a procedural comparison. *Epidemiology Biostatistics and Public Health*. 2016; 13, 3, e11734-1/e11734-7.
- Visalli G, Facciola A, Laganà P, et al. Food chemoprevention and air pollution: the health comes with eating. *Rev Environ Health*. 2020 Jun 22. doi: 10.1515/reveh-2019-0072.
- Rousham EK, Unicomb L, Islam MA. Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches. *Proc Biol Sci*. 2018;285(1876): 20180332.
- European Food Safety Authority. Report of the Task Force of Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers. *EFSA Journal*, 2007, 96:1–46 (<http://www.efsa.europa.eu/fr/efsajournal/doc/96r.pdf>).
- Facciola A, Riso R, Avventuroso E, et al. *Campylobacter*: State of art at 2015. *J Prev Med Hyg* 2017; 58: E79-E92. Cod. Scopus 2-s2.0-85021816739
- Marti R, Scott A, Tien YC et al. Impact of Manure Fertilization on the Abundance of Antibiotic-Resistant Bacteria and Frequency of Detection of Antibiotic Resistance Genes in Soil and on Vegetables at Harvest. *Appl Environ Microbiol*. 2013; 79(18):5701–5709.
- Hölzel CS, Tetens JL, Schwaiger K. Unraveling the Role of Vegetables in Spreading Antimicrobial-Resistant Bacteria: A Need for Quantitative Risk Assessment. *Foodborne Pathog Dis*. 2018 Nov 1; 15(11): 671–688.
- Bauer AW, Kirby WM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45:493–496.
- Survey of healthcare workers' knowledge, attitudes and behaviours on antibiotics, antibiotic use and antibiotic resistance in the EU/EEA, 2019, doi: 10.2900/099807).
- Antibiotic resistance and use of antibiotics in Tuscany, Report 2016, Editing: Baldocchi C - ARS Tuscany, Italy).
- Montagna MT, Mascipinto S, Pousis C, et al. Knowledge, experiences, and attitudes toward Mantoux test among medical and health professional students in Italy: a cross-sectional study. *Ann Ig* 2018; 30 (Suppl. 2): 86-98.
- Bonville CA, Domachowske BJ, Cibula DA, Suryadevara M. Immunization attitudes and practices among family medicine providers. *Hum Vaccin Immunother*. 2017 Nov; 13(11): 2646–2653.

20. Martins C, Norsett-Carr A. Obesity Knowledge among Final-Year Medical Students in Norway. *Obes Facts*. 2018 Feb; 10(6): 545–558.
21. Cox G, Wright GD. Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions *International Journal of Medical Microbiology*, 2013, 303 (6–7): 287–292.
22. Kihyun L, Dae-Wi K, Do-Hoon L, et al. Mobile resistome of human gut and pathogen drives anthropogenic bloom of antibiotic resistance. *Microbiome*. 2020; 8: 2. Online 2020 Jan 7. doi: 10.1186/s40168-019-0774-7
23. von Wintersdorff CJH, Penders J, van Niekerk JM, et al. Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Front Microbiol* 2016;7:173.
24. Walsh FM, Amyes SGB. Microbiology and drug resistance mechanisms of fully resistant pathogens. *Current Opinion in Microbiology* 2004; 7(5): 439–444.
25. Peterson E and Kaur P. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Front. Microbiol.*, 30 November 2018 <https://doi.org/10.3389/fmicb.2018.02928>.
26. Wright G. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat Rev Microbiol* 2007; 5: 175–186 <https://doi.org/10.1038/nrmicro1614>.
27. Blau K, Bettermann A, Jechalke S, et al. The transferable resistome of produce. *mBio* 9:e01300-18. 2018. <https://doi.org/10.1128/mBio.01300-18>. Editor Mark J. Bailey, CEH-Oxford.
28. MacFadden DR, McGough SF, Fisman D, et al. Antibiotic resistance increases with local temperature. *Nature Climate Change*, 2018;8:510–514.
29. Bengtsson-Palme J, Kristiansson E, Larsson DGJ. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiol Rev*. 2018 Jan; 42(1): fux053.
30. Chng KR, Li C, Bertrand D, et al. Cartography of opportunistic pathogens and antibiotic resistance genes in a tertiary hospital environment. *Nat. Med*. 2020; 26(6): 941–951.
31. Diwan V, Hanna N, Purohit M, et al. Seasonal Variations in Water-Quality, Antibiotic Residues, Resistant Bacteria and Antibiotic Resistance Genes of *Escherichia coli* Isolates from Water and Sediments of the Kshipra River in Central India. *Int J Environ Res Public Health*. 2018;15(6): 1281.
32. Ivanov AV, Bartosch B, Isaguliantz MG. Oxidative Stress in Infection and Consequent Disease. *Oxid Med Cell Longev*. 2017:3496043.
33. Casella S, Ielati S, Piccione D, et al. Oxidative stress and band-3 protein function in *Liza aurata* and *Salmo irideus* erythrocytes: effect of different aquatic conditions. *Cell Biochem Funct*. 2012;30(5):406–10
34. Coniglio MA, Laganà P, Faro G, et al. Antimicrobial Potential of Sicilian Honey against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J AOAC Int*. 2018 Jul 1;101(4):956–959.
35. Laganà P, Anastasi G, Marano F, et al. Phenolic Substances in Foods: Health Effects as Anti-Inflammatory and Antimicrobial Agents. *Journal of AOAC International*, 2019: 102 (5), 1378–1387.
36. Mancuso M, Catalfamo M, Laganà P et al. Screening of antimicrobial activity of citrus essential oils against pathogenic bacteria and *Candida* strains. *Flavour and Fragrance Journal*, 2019; 34(3):187–200.
37. Overbey KN, Jaykus LA, Chapman BJ. A Systematic Review of the Use of Social Media for Food Safety Risk Communication. *J Food Prot* (2017) 80 (9): 1537–1549.
38. Mania I, Barone C, Pellerito A, et al. Transparency and promotion of food productions. Labelling and traceability in the food supply chain as defense instruments for food products. *Ind. Alim*. 2017; 56(581):18–22.
39. Regan A, Raats M, Shan LC, Wall PG, McConnon A. Risk communication and social media during food safety crises: a study of stakeholders' opinions in Ireland. *Journal of Risk Research*, 2016; 19(1): 119–133.
40. Flynn K, Pérez Villarreal B, Barranco A et al. An introduction to current food safety needs. *Trends in Food Science & Technology* 2019; 84:1–3.

Correspondence:

Pasqualina Laganà

Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy.

E-mail: [plagana@unime.it](mailto:plagana@unime.it)