JOURNAL OF INTERNATIONAL CRISIS AND RISK COMMUNICATION RESEARCH
ISSN: 2576-0017
2024, VOL 7, NO S9

Concentration and Residual Activity of Expired Gentamicins

Mex-Álvarez Rafael Manuel de Jesús, Guillen-Morales María Magali, Garma-Quen Patricia Margarita, Ferrer-Dzul Dylan Manuel, Chan-Martínez Roger Enrique, Euan-García Daniel Eduardo

1. Drug Analysis Laboratory of the Pharmacy Area of the Faculty of Chemical-Biological Sciences of the Autonomous University of Campeche.

Email: rafammex@uacam.mx

ABSTRACT

Medicines are currently considered emerging contaminants due to a deficiency in their final disposal and the lack of adequate treatments for their elimination in wastewater; This causes environmental damage by altering the physiology of microorganisms, plants and animals exposed to expired or improperly discarded medications; One of the groups of most interest for study are antibiotics because they can cause public health problems such as the generation of resistance by microorganisms. The objective of this work was to evaluate the residual and minimum inhibitory concentrations (MIC) of gentamicin in expired drugs versus Escherichia coli and Bacillus clausii to estimate their ecopharmacological impact; To do this, the active ingredient was quantified using the ninhydrin technique and the MIC was determined using the well microdilution technique. The results show that after 120 months of expiration it is still possible to detect active ingredient (close to 50%) in the medications and that they retain significant antibiotic activity capable of affecting the environment (MIC from 8 to 512 ▫g/mL). ; For this reason, it is important to promote health education that promotes correct final disposal of expired medications to avoid damage to public and environmental health.

KEYWORDS: Gentamicin, expired medications, residual concentration, ecopharmacological impact, antimicrobial resistance

1. Introduction

Although medicines are important and indispensable goods in modern societies that contribute to improving the quality of life; The abuse of its consumption represents a danger to public health both by the appearance of adverse reactions and by causing environmental pollution after its inappropriate disposal through different routes such as municipal solid waste, toilets or throwing it on the ground [1, 2]. Even in low concentrations, drugs or active ingredients of medicines wreak havoc on ecosystems by altering the physiology of microorganisms, plants and animals and by direct exposure or through the food chain they affect human beings; This is demonstrated by scientific studies carried out since the 1970s in the United States in which the

presence of antihypertensives, antibiotics, analgesics, antiepileptics and antidepressants in the environment was reported through different routes of entry into ecosystems such as incorrect disposal. from medications or from untreated hospital water discharges [3-5].

Of the pharmaceutical groups found in the environment, antibiotics have received special interest due to the scope of their harmful effects since their presence in soil and water alters the microenvironments of ecosystems, alters the ecological balance of microorganisms, favors the appearance of antibiotic-resistant bacteria [4-7]. The groups of antibiotics most used in clinical practice are quinolones, macrolides, penicillins and aminoglycosides; In general, the effect of these chemical substances on the environment and on different organisms is unknown; It is also not well elucidated how it can affect human health when foods contaminated with antibiotics are consumed [6,7].

During a campaign to collect expired medicines carried out in the City of San Francisco de Campeche, with the purpose of raising awareness in the population through health education, a large amount of aminoglycosides were obtained; These are antibiotics that act by altering protein synthesis by binding to the 30S subunit of the bacterial ribosome and that are used in pharmacotherapy to treat urinary infections caused by gram-negative bacteria and due to their chemical structure, they are very soluble in aqueous media and have great stability in the environment. environment, which complicates its removal in contaminated waters and aminoglycosides have been found in hospital effluents after wastewater treatment [8,9]. Consequently, the objective of this work was to determine the concentration and residual antibacterial activity of gentamicin present in expired medications to estimate its ecotoxicological impact due to its poor disposal of these antibiotics.

2. Methodology.

Expired medications collected during campaigns organized in the 2022-2023 biennium by the Drug Information Center of the Pharmacy Area of the Faculty of Chemical Biological Sciences of the Autonomous University of Campeche were selected. The inclusion criteria were: expired medications with at least two vials from the same batch that had their secondary packaging in good condition. The batch, dose, manufacturer and expiration date of the medications included in the study were recorded.

Spectrophotometric quantification of the active ingredient [10-13].

The residual quantification of the active ingredient contained in the expired medications was carried out by a colorimetric technique using 1.25% ninhydrin as a color developing reagent. After heating in a boiling water bath for 15 minutes, the reaction tube was placed in a water bath. ice and measured in the spectrophotometer at 540 nm, each quantification was performed in triplicate, a calibration curve was performed with standard glycine solutions.

Determination of residual antibacterial activity [14-16].

To determine the minimum inhibitory concentration (MIC), the microtiter technique was used in sterile 96-well plates; the culture medium used was Müeller Hinton Broth. Initially, 50 mL of culture broth at double concentration was placed in each well, later it was mixed with 50 mL of antibiotic to make a serial 1:2 dilution, 50 mL of the mixture was eliminated in the last well to have the same volume in all the wells; Then, the corresponding inoculum (E. coli ATCC 25922 or Bacillus clausii reactivated from Enterogermina ®) with turbidity comparable to Mc Farland's 0.5 standard, read on a digital nephelometer (106-108 CFU/mL), was added. The microplates were incubated for 24 h at 37ºC and subsequently 10 □L of a 0.25% MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was added and incubated again. at 37ºC for 20 more hours. Once the incubation time was over, the results were read visually, determining the well with the highest dilution that inhibited bacterial growth (absence of the dye vire, colorless).

3. Results and discussions.

Graph 1 shows the residual concentrations of the active ingredient in the injectable solutions of gentamicin, this value is contrasted with the expiration time of the medication; It can be seen that there is a tendency to decrease the concentration of the active ingredient proportionally with the expiration time; the gentamicin with the longest expiration time (120 months) had a concentration corresponding to 48% of that indicated for the original medication. Although there is a relationship between these variables, there are other factors that can affect the residual concentration of the drug. This can be observed in medications with a similar expiration time but that present differences in their residual concentration; This is because the medications had different conditions during their storage and handling by the users since many patients do not have first aid kits or exclusive places to protect their medications or they deposit them in unsuitable places exposed to humidity, light or heat [17,18].

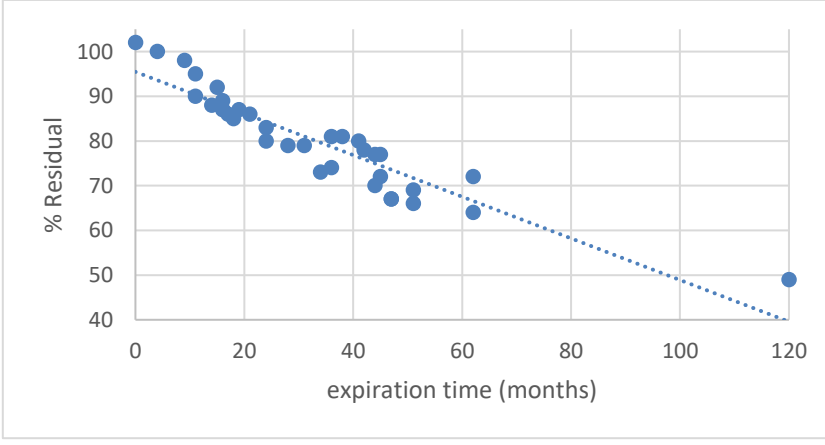

Figure 1. Percentage of residual active ingredient of expired gentamicins with respect to expiration time.

The decomposition of the active ingredient of an expired medication is a case analogous to its metabolism because it does not necessarily correspond to its biological activity; since the metabolite may retain part of its chemical structure that is quantified (in this case it would be that the degradation compounds retain some amino groups that react with ninhydrin) but do not have antibacterial action. The case may also be presented that the biological activity is maintained because compounds formed from the degradation of the active ingredient retain their bioactivity; Therefore, the evaluation of residual bioactivity is a step that cannot be ignored [19,20]. In this same sense, subsequent studies should continue with the evaluation of the toxicity of expired medications to rule out that during their decomposition, compounds more toxic than the original active ingredient are not produced.

In this case, a loss of antibacterial activity proportional to the decrease in the concentration of the active ingredient was observed. Figure 2 shows that the MIC of the expired drugs increased for both microorganisms evaluated; E. coli was more sensitive to the drug compared to Bacillus clausii, the drug with the least antibacterial activity was the oldest (120 months of expiration) which had an MIC of 256 mg/mL for E. coli and 512 mg/mL. mL for B. clausii compared to the non-expired drug which had an MIC, for both bacteria, of 8 mg/mL.

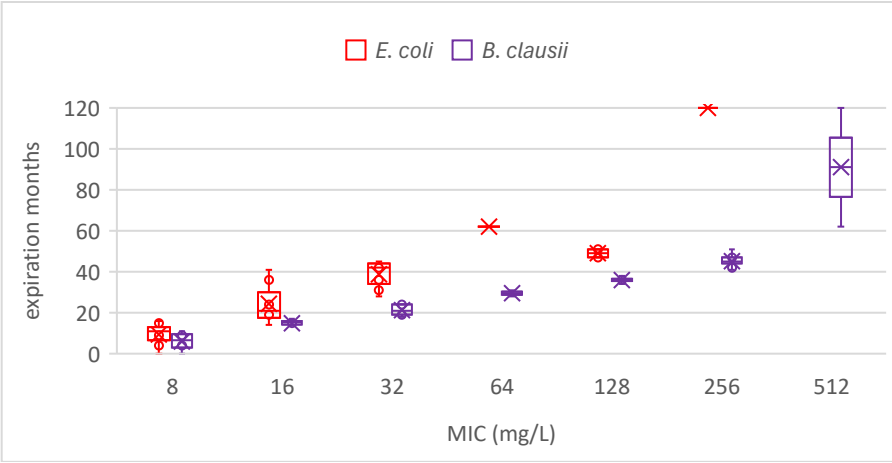

Fig. 2. Minimum inhibitory concentrations of Escherichia coli and Bacillus clausii by expired gentamicins with respect to their expiration time.

Escherichia coli is a gram-negative bacteria that can be considered opportunistic because it is usually part of the intestinal microbiota without causing harm to humans but can cause infections in certain situations; Being part of the intestinal microbiota, it is exposed to different antibiotics administered to a patient and therefore has developed multiple resistance mechanisms to different antibiotics; Therefore, this bacteria was chosen as a monitor for residual antibiotic activity due to its clinical relevance [21].

The selection of Bacillus clausii was to estimate the environmental impact of expired medications, since this gram-positive bacteria is found in soils and, like other species

of the same genus, promotes plant growth by inducing the solubilization of phosphorus species, producing regulators of growth, participate in nitrogen fixation, control pests and diseases; For all this, it constitutes a good environmental monitor to estimate the ecological impact that the presence of expired medications in the soil would cause. Likewise, B. clausii is used clinically as a probiotic; in fact, the certified strains used in the trials were reactivated from a medication that exclusively contains spores of this bacteria that is indicated for the treatment of diarrhea caused by alterations in the intestinal microbiota [22 ,2. 3].

The antibacterial spectrum of gentamicin is mainly against gram-negative bacteria, although it also presents activity against gram-positive bacteria [24,25]. This coincides with the MIC values obtained and explains why E. coli was more sensitive to expired drugs compared to B. clausii. The residual activity corresponds to the MIC, which rules out the possibility that the compounds generated during the degradation of the drug have antibacterial activity; Therefore, the importance of promoting an environmental culture through continuous health education of communities so that they become aware that the expiration of a medicine does not mean the loss of biological activity nor does it represent environmental safety. In this sense, it is recommended to continue with the ecotoxicological evaluation of expired gentamicins to rule out that the degradation products are not more toxic compared to the original active ingredient.

These initial studies represent the scientific basis for understanding the ecological impact of medicines on the environment and consequently represent a contribution to the ecopharmacovigilance of antibiotics with a view to preventing their adverse effects on plant, animal and human health. [26,27]. The medications evaluated in this study come from various campaigns to collect expired medications in different communities. Several tons of these have been collected and antibiotics are a very important group, due to their quantity, in the recovered medications; If they had not been collected from homes for proper disposal by the company providing the service, these antibiotics would have been disposed of by various means such as the municipal garbage collection service, discarded in bins or toilet bowls, or dumped on the ground with the consequent environmental pollution [26-27].

4. Conclusion.

Expired medications containing gentamicin retain more than 50% of their active ingredient and antimicrobial activity until 120 months after expiration, so poor final disposal of these medications continues to represent a serious risk to environmental and human health because In contact with bodies of water that contain human pathogenic bacteria such as Escherichia coli, they can induce the generation of resistance to antibiotics, or in contact with soils, they can alter their ecological balance by killing beneficial microorganisms such as Bacillus clausii, favoring the growth of pathogenic species.

References

Sánchez-Ramírez, J. E. (2018). Contaminantes emergentes en nuestros ecosistemas: enemigos invisibles. Ingeniería y Región, 19, 18-21.

García-Gómez, C., Cortáres-Moroyoqui, P. & Drogui, P. (2011). Contaminantes emergentes: efectos y tratamiento de remoción. Química Viva, 10 (2), 96-105.

Moreno-Barragán, A. S., Benalcázar-Pozo, C. A., & Bermúdez-del Sol, A. (2023). Contaminación ambiental por productos farmacéuticos y su impacto en la salud humana. Revista de Ciencias Médicas de Pinar del Río, 27.

Tejada, C., Quiñonez, E., & Peña, M. (2014). Contaminantes emergentes en aguas: metabolitos de fármacos. Una revisión. Revista Facultad de Ciencias Básicas, 10(1), 80-101.

Gil, M. J., Soto, A. M., Usma, J. I. & Gutiérrez, O. D. (2012). Contaminantes emergentes en aguas, efectos y posibles tratamientos. Producción + limpia, 7(2), 52-73.

Amé, M. V., Bertrand, L., & Valdés, M. E. (2023). Antibióticos ¿contaminantes de preocupación emergente en Córdoba? antibióticos en sistemas acuáticos. Bitácora Digital, 10(14), 91-98.

Becerril Bravo, J. E. (2009). Contaminantes emergentes en el agua. Revista Digital Universitaria, 10(8).

Sermet, K. & Alfandari, S. (2021). Aminoglucósidos. Tratado de medicina, 25(4), 1-4.

Chávez Vela, N. A., Jáuregui Rincón, J., Araiza Arvilla, J., & Montañez Flores, A. L. (2022). Detección de antibióticos aminoglucósidos en plantas de tratamiento de aguas residuales mediante Dot blot. Ciencia Transdisciplinar en la Nueva Era, 207.

Suárez-Torres, G., & Soledad, B. (2021). Desarrollo de un método analítico para determinar amikacina mediante Polímeros de Impronta Molecular combinados con Extracción en Fase Sólida (MISPE).

Raut, A., Sharma, D., & Suvarna, V. (2022). A status update on pharmaceutical analytical methods of aminoglycoside antibiotic: amikacin. Critical Reviews in Analytical Chemistry, 52(2), 375-391.

Usmani, M., Ahmed, S., Sheraz, M., & Ahmad, I. (2018). Analytical Methods for the Determination of Amikacin in Pharmaceutical Preparations and Biological Fluids: A Review. Iranian Journal of Analytical Chemistry, 5(2), 39-55.

González Guerrero, R., Hernández, A., Cantú Cárdenas, L. G., & Esquivel Ferriño, P. C. (2018). Estudio cinético de la liberación in vitro de gentamicina desde un biomaterial cerámico.

Paredes, F., & Roca, J. J. (2004). Acción de los antibióticos. Offarm, 23(3).

Pascual, I. P., Parra, J. C., & Concha, V. M. T. (2022). Tratamiento antibiótico. Medicine-Programa de Formación Médica Continuada Acreditado, 13(49), 2853-2863.

Horna Quintana, G., Silva Díaz, M., Vicente Taboada, W., & Tamariz Ortiz, J. (2005). Concentración mínima inhibitoria y concentración mínima bactericida de ciprofloxacina en bacterias uropatógenas aisladas en el Instituto Nacional de Enfermedades Neoplásicas. Revista Medica Herediana, 16(1), 39-45.

Vargas, E. C., Cruz, W. L., Hernández, A. E., & Alvarado, D. M. (2021). Buenas prácticas de almacenamiento de medicamentos en el Hospital Escuela de Tegucigalpa, Honduras. Journal of Pharmacy & Pharmacognosy Research, 9(5), 563-572.

Alegría Aldana, L. M. (2021). Estudio de la composición, almacenamiento y prácticas de uso del botiquín familiar en estudiantes de la Licenciatura en Química Farmacéutica de Guatemala.

Sánchez, J. E., Pastor, L., Doñate, S., & Claros, J. (2019). Evaluación de la degradación de contaminantes emergentes en el proceso de digestión anaerobia de lodos de EDAR. Novedar,(June).

Del Arco, C. (2008). Metabolismo de los fármacos. Farmacología humana, 5, 73-85.

Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). Escherichia coli in Europe: an overview. International journal of environmental research and public health, 10(12), 6235-6254.

João Alves, Lex, Wisoczynski de Sene, Danielly, Ferreira de Paula, Gabriel, Bueno Demétrio, Gilberto, & Sussumu Matsumoto, Leopoldo. (2021). Influencia de Bacillus sp. sobre los atributos químicos y microbiológicos del suelo y el desarrollo de la soja y el maíz. Revista mexicana de ciencias agrícolas, 12(3), 383-393.

Lee, N. K., Kim, W. S., & Paik, H. D. (2019). Bacillus strains as human probiotics: characterization, safety, microbiome, and probiotic carrier. Food science and biotechnology, 28(5), 1297–1305.

Díaz Q, Patricia, Bello T, Helia, Domínguez Y, Mariana, Trabal F, Natalia, Mella M, Sergio, Zemelman Z, Raúl, & González R, Gerardo. (2004). Resistencia a gentamicina, amikacina y ciprofloxacina en cepas hospitalarias de Klebsiella pneumoniae subespecie pneumoniae productoras de ß-lactamasas de espectro extendido. Revista médica de Chile, 132(10), 1173-1178. https://dx.doi.org/10.4067/S0034-98872004001000003

glesias Lamas, E., Conde Rivera, O., Santos Nores, J., & Borrajo Prol, M.P.. (2011). Valoración de la resistencia a gentamicina tras su uso diario en curas de catéteres peritoneales. Nefrología (Madrid), 31(5), 613-614.

Barrantes Jiménez, Kenia, Chacón Jiménez, Luz, & Arias Andrés, María. (2022). El impacto de la resistencia a los antibióticos en el desarrollo sostenible. Población y Salud en Mesoamérica, 19(2), 305-329. https://dx.doi.org/10.15517/psm.v0i19.47590

Rosenblatt-Farrell, Noah. (2009). El paisaje de la resistencia a los antibióticos. Salud Pública de México, 51(5), 435-442.