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Evolution of Antimicrobial Resistance of the Main Causative Agents of Nosocomial Infection

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Abstract

Introduction: Nosocomial infections constitute a global sociobiological process from local cases, to be passed in all hospital institutions and by their similar etiology, and through the evolution by natural selection of their causal agents, in which their development of resistance to antimicrobials is vital. This perspective is necessary in its epidemiological surveillance in order to detect in a timely manner changes in pathogenic hospital microbiome and in its sensitivity and resistance to antimicrobials and to formulate more effective strategies for the control, treatment and abatement of its incidence.

Methodology: With the objective of showing possible evolutionary changes of the causal agents of nosocomial infections, regarding its virulence, pathogenicity and antimicrobial resistance; in a hospital with 300 number of census beds and 10,000 annual patient discharges, a clinical-epidemiological, descriptive, longitudinal and comparative study was conducted regarding nosocomial infections, their causative agents and their resistance to antimicrobials during the years 2014 to 2020. Through active epidemiological vigilance, with the aim of identifying and confirming probable cases of nosocomial infection in a clinical, microbiological and epidemiological manner; main nosocomial infections and their causative agents were selected and tested again statin microbials with automatic microbiology equipment for cultures and antibiogram.

Results: In this period, although the main nosocomial infections (pneumonia, of urinary tract, surgical site and bacteremia) and, likewise, their causal agents (*Acinetobacter baumannii, Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) remained unchanged; all did so through changes between such infections that evidence inter bacterial competition, even between different strains of the same species and, in general, expanded the sites of infection, their incidence and the global resistance to antimicrobials, although with ups and downs and contradictions.

Conclusion: The increase in morbidity and changes in the epidemiological profile of nosocomial infections show the development of virulence and pathogenicity of their causative agents, correlated with the development of antimicrobial resistance. And none of it could happen without adjustments to their genomes. All these evolutionary changes do not occur in a linear way, but with ups and downs and contradictions. This information has been useful to know the trend of pathogenic bacterial microbiome in the Hospital and to specify a rational use of antibiotics to combat it.

Keywords: Nosocomial infections; Bacterial evolution; Antimicrobial resistance

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Introduction

Nosocomial Infections (NI) are constituted and developed by a global socio-biological process that is dialectically concretized in particular local cases; because all hospitals affected are social institutions which structure, organization, administration, functioning and operation determine a specific ecological niche in which a particular microbiome develops, differing from the environment of the community and the nature wherein it is located. On the one hand, factors favorable to the colonization, reproduction, transmission and contagion of pathogenic germs are concentrated in hospitals: The most serious infections, the patients most susceptible to them, a constantly controlled environment in terms of temperature, humidity, ventilation, etc., effective means of direct and cross-transmission, due to individual contact between patients and health personnel and, above all, to invasive procedures for diagnosis and treatment (punctures, venoclysis, catheters, tubes, endoscopies, intubations, surgery, etc). And on the other hand, because the measures and substances used extensively for the prevention (cleaning, hygiene, asepsis and antisepsis) and the treatment

of infections, with the predominance of antimicrobials, radically modify the hospital microbiome; denaturing it, causing dysbiosis and opening spaces for colonization by pathogenic germs [1,2]. In the particular case of the Regional General Hospital 1 (HGR 1), the site for this study, an institutional program for the prevention and control of infections associated with health care is carried out [3]; through 17 processes of priority attention for the epidemiological surveillance of NI, cleaning and disinfection, correct hand hygiene, good practice for invasive procedures, sterilization and equipment, antiseptics, isolation precautions, biological-infectious residues, etc. Of the immense number of microbial species, "those that produce infectious diseases in humans are less than one hundred" [4]. In any given environment, an absolute majority of non-pathogenic microorganisms occupy its space and, thus, "a diverse microbiome creates with its very presence a barrier against disease, a resistance to colonization" by pathogens. Consequently, a paradox occurs, "toilets that are cleaned too often are more likely to be covered with fecal bacteria and asepsis, when taken too far, can make diversity disappear"; so, "the zeal to sterilize hospitals has created dysbiosis" [5]. When combat against microorganisms is more avid, integral and powerful, it modifies and decreases to large extent the original microbiome, causing a natural selection and promoting the development of the most empowered microorganisms against such health and medical resources; as has happened with the superbugs of the acronym ESKAPE: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. [6,7].

The disease etiology by microorganisms depends fundamentally on three correlated factors: Their virulence and pathogenicity and their response to antimicrobials. Virulence is the quantitative aspect of pathogenicity (the particular mode of acting on a morbid cause on an organism), measured by the number of microorganisms needed to cause an infectious-contagious disease in a susceptible host. It is determined by multiple virulence factors, which act individually or jointly during the different stages of infection [8]. And when it is associated with health care, the virulence and pathogenicity also depend on the response of germs to preventive measures (cleaning, hygiene, asepsis, antisepsis, vaccination) and therapeutic measures, mainly antimicrobials.

Since, as Theodosius Dobzhansky has pointed out, "nothing in biology makes sense except in the light of evolution", also in the case of biological factors in the incidence of nosocomial infections this theory illuminates its processes to make them understandable and concretize its scientific explanation [9]. Charles Darwin synthesized thus in his theory of evolution.

As many more individuals of each species are born than can possibly survive; and as, consequently, there is a frequently recurring struggle for existence, it follows that any being, if it vary however slightly in any manner profitable itself, under the complex and sometimes varying conditions of life, will have a better chance of surviving, and thus be naturally selected. From the strong principle of inheritance, any selected variety will tend to propagate its new and modified form" [10,11].

Then, as Ed Yong put it: "Evolution through natural selection depends on exactly three things: individuals must vary, these variations must be heritable, and these variations must have sufficient potential to determine their fitness; that is, their ability to survive and reproduce. Variation, heredity and fitness" [5]. Although such an evolutionary process takes place for very long periods of time in multicellular beings, their very high and accelerated reproduction rates of microorganisms and very short life expectancy together drive a very rapid evolution in them. In the particular case of bacteria, based on a refined experiment through 50 thousand generations of [12] populations of *Escherichia* coli, Richard Lenski identified three fundamental factors inter-related in their evolutionary process: Genetic variation, divergence of strains, species and populations, and adaptation to their colonized environment [11].

With regard to infectious-contagious diseases, "bacteria, throughout evolution, have acquired... factors or determinants of virulence... that favor their growth or survival during infection"; which are activated in two phases: An early one to promote colonization and invasion of the host (adhesion, mobility and chemotaxis, invasion); and a late one, properly pathogenic, to develop self-defense mechanisms (survival and intracellular mobility, evasion of the immune response and antigenic variation, subjugation and confrontation);with differences between hospital and community [12].

For his part, Richard C. Lewontin has specified the dialectic of the inter relationship germen/environment: "It is neither genes nor the environment which determine an organism, but a particular combination of both the organism depends on both genes and the environment... organisms are not only the product, but also the creators of their own environments... It is not simply an interaction of internal and external factors, but also a dialectical development of the organism in response to its mutual influence". Consequently, "a genotype does not give rise to a single type of development, but to a reaction norm, a scheme of different types of development in different environments" and, likewise, "different genotypes will evolve differently in the same environment" [13]. In this way, genes (the genotype) not only determine an organism (the phenotype), they also indirectly influence its environment, making it an extension of its phenotype [5].

Therefore, this correlation is the determinant of particular and different hospital infectious processes that occur in community and nature; thanks to four main factors: (1) "organisms determine which elements of the outside world will constitute their environment" and which of them are relevant to relate; (2) thus, they "continuously alter their environment"; (3) even "actively build the world around them"; (4) and with all this, "external conditions become part of the environment of the organism" [14].

In this context, the evolution of a living being requires two main resources: Genotypes co-adapted to their cohabitants (in a parasitic or mutualistic way) and to their environment, and to compete with them in an arms race. In principle, "the process of adaptation by natural selection requires the genetic variation of those characteristics that influence the survival and reproduction of organisms"; which occurs by de-novo mutation (change of locus of a point gene, by rearrangement or transposition) or by admixing (mixis: the production of a new genotype by recombination, conjugation, transduction or transformation). Secondly, individual genetic variation (by genetic exchange including interspecies, changes on its own genes or reactivation of their encrypted genes)can also cause phenotypic changes and the development of new metabolic functions; which causes a divergence in the population to which it belongs (by transient polymorphism, selective neutrality or frequency-dependent selection) and the emergence, eventually, of new species strains [11]. But ultimately, the evolution of an organism involves its insertion and

adaptation or adequacy to the environment (fitness). Genes only work when there is some structure on which they can act. "They are not selected for their intrinsic qualities, but by virtue of their interactions with the environment... and the most important environmental part is perhaps the other genes they encounter... The genes themselves do not evolve, they only survive or not in the gene pool. It is the 'team' that evolves in cooperation towards solutions to different problems", environmental stimuli and stressors [14].

The "arms race", on the other hand, is "the most satisfactory explanation for the existence of the advanced and complex machinery possessed" by living beings. Between members of the same or different species there is a struggle for existence, in order to obtain limited vital resources, survive and reproduce, in the face of a competitive and hostile environment. Thus, those with the best "weapons" to compete and defend themselves against the environmental aggressions are more likely to survive and thrive. However, up to a certain limit, when those become too metabolically costly for their production and operation [11]. This is the case with antimicrobial resistance mechanisms, which are capable of being activated, acquired and developed by bacteria and which have become a global problem for public health.

According to the World Health Organization (WHO): "Antibiotic resistance is a growing threat to public healthcare around the world. Infections caused by pathogens resistant to them substantially increase the burden of both health-care-associated infections and community-acquired infections" [15]. However, it is particularly serious in the case of nosocomial infections, since they constitute "the most frequent adverse event in hospital care", since "a greater resistance of microorganisms to antimicrobials" is observed here [5]. This has been fully demonstrated, for example, in the case of urinary tract infections [16].

As has been shown, "the development of generations of antibiotic-resistant microbes and their distribution among microbial populations throughout the biosphere are the result of many years... of the use of antibiotics, to under or overdose or inappropriately... there is perhaps no better example than this of darwinian notions of selection and survival" [17]. And in this regard the WHO pointed out: "This type of resistance can result from a species-wide characteristic or occur between strains of species that are usually sensitive, but develop resistance by mutation or gene transfer. Resistant genes encode several mechanisms by which microorganisms can resist the inhibitory or neutralizing effects of specific antimicrobial agents. Such mechanisms also generate resistance to other antimicrobials of the same class and sometimes too many compounds of different classes" [18]. In fact, "the spread of antibiotic-resistant bacteria is one of the greatest threats to public health in the twenty-first century and a testament to the immense power of horizontal gene transfers" between them [5].

Antimicrobials and antibiotics are substances that attack certain bacterial vital processes; especially those which use different enzymes or structures, absent or uncommon in eukaryotic cells. There are four main classes, according to their targets of attack: Cell wall (β-lactam and non-lactam), protein (aminoglycosides, tetracyclines, lincosamides, linezolides, etc.), nucleic acids (fluoroquinolones), and folate synthesis (sulfonamides). Such effects and targets characterize and differentiate antibiotics and, concomitantly, define resistance to them [19,20].

On the other hand, bacteria resist antimicrobials due to various structural mechanisms (chromosomal) or acquired (by mutation, conjugation or gene transfer); being five the fundamental against these drugs: (1) inactivation or alteration by enzymes; (2) modification of the target sites for its action (by loss of porins, efflux pumps, etc.); (3) changes in the outer membrane permeability to reduce intracellular accumulation; (4) mutation of DNA polymerases; and (5) the formation of biofilms [7, 21,22].

Therefore, and in summary, the evolution of a microorganism can be observed in a direct way in the modification during a certain time of its genome and its biochemical products: by sequencing the DNA and "also the RNA, the proteins and the metabolites; DNA reveals what microbes there are and what they are capable of doing, and the other molecules say what they actually do" [5]. And indirectly, its evolution is manifested in the changes in its virulence, pathogenicity and, specifically, its resistance to antimicrobials; which does not take place in a unidirectional or linear way, but as uneven, with ups and downs and contradictions.

Materials and Methods

With the objective of evidencing possible evolutionary changes of the causative agents of nosocomial infection, regarding their virulence, pathogenicity and resistance to antimicrobials; in the Regional General Hospital 1 (HGR 1), with 300 censurable beds and 10,016 patient discharges in 2020, a descriptive, longitudinal and comparative clinical-epidemiological study was conducted regarding nosocomial infections, their causative agents and their resistance to antimicrobials during the years 2014 to 2020. From the initial medical diagnosis, by means of an active and daily epidemiological surveillance of all hospitalized patients, with the team of an epidemiologist, three specialist nurses and the Microbiology Laboratory we identified, studied and confirmed suspected cases of nosocomial infection in a clinical, microbiological and epidemiological manner. Of the total, we selected the main and predominant nosocomial infections occurred; being four: pneumonia, urinary tract, surgical site and bacteremia. In every case relevant samples were taken (secretions, sputum, urine, blood), in order to realize cultures and antibiogram; applying "the procedures for taking, handling and shipping of biological specimen for confirmatory diagnosis of conditions subject to epidemiological surveillance, with the purpose to ensure that they are met the optimum conditions" for its analysis with reliability and validity, according to the protocol of the institutional Manual for the Laboratory-based epidemiological surveillance [23]. For that purpose, it was utilized an Biomérieux Vitek® 2 automatized equipment, which uses a card system with colorimetric reagents inoculated with a suspension of pure microbial culture, and the developed profile is automatically interpreted with the identification of different classes of organisms: Gram-negative fermenting and non-fermenting bacilli, Gram-positive non-spore forming coccus and bacilli, yeast and yeast-like organisms and Gram-positive spore-forming bacilli.

Among the total causal agents identified of the four main and predominant nosocomial infections, the most frequent ones were also selected; being five: *Acinetobacter baumannii*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. And after differentiating the min the infections they caused, we tested in all them the four classes of antimicrobials, according to the synthesis neutralization prompted: Cell wall, proteins, nucleic acid (DNA topoisomerases) and folates; in total, 26 antimicrobials against Gram-negative and 15 against Gram-positive. And at last, for the results analysis we resorted to descriptive and inferential statistics (χ^2,

odds ratio and *p*).

Results

In a previous study [24], a total of 1,079 nosocomial infections(with a rate of 8 per 100 discharges) were detected at HGR 1; four of them stand out for their higher frequency: Bacteremia (21%), surgical site (20.2%), pneumonia (17.3%) and urinary tract (7.5%); these four adding up to two thirds of the total (66%). In 300 of all cases (27.8%), culture and antibiogram tests were performed; identifying 33 species of microorganisms, mostly bacteria (94.8%) and a smaller part were yeasts (*Candida albicans* and sp: 5.2%). Bacteria were mostly Gram-negative (60.9%) and more than a third Gram-positive (39.1%); among them five stood out, exceeding two thirds of the total (67.2%): *Staphylococcus aureus* (20%), *Escherichia coli* (19.3%), *Pseudomonas aeruginosa* (15.3%), *Acinetobacter baumannii* (7.3%) and *Klebsiella pneumoniae* (5.3%). Against them we tested 34 antimicrobials of the four classes and, although with variations depending on the type of antibiotic and also site of infection, all main bacteria were multidrug-resistant to them.

For this one, a second study was carried in the same HGR 1 for comparison with the previous one during the year 2020, among 10,016 hospital patients 812 NIs were detected (with a rate of 8.1 NI per 100 discharges); in its great majority (80.7%) by four main infections: Pneumonia (35.5%), urinary tract (17.1%), bacteremia (15.1%) and surgical site (13%). From the total of NIs, 488 cultures and antibiograms were performed (60.1%); identifying 44 species of microorganisms, with a majority of bacteria (86.1%), more Gram-negative (54.7%) than Gram-positive (31.4%), and a minority of yeasts (13.9%). Of the bacteria, five (59%) stoodout: *Acinetobacter baumannii* (15.8%), *Staphylococcus aureus* (14.5%), *Escherichia coli* (13.7%), *Pseudomonas* aeruginosa (7.6%) and *Klebsiella pneumoniae* (7.4%). And for the yeasts, *Candida albicans* (10%). The incidence of NIs varied each year, although the four main ones remained on place, which increased their participation from 64.6% in 2014 to 85.2% in 2017. During the six-year period studied those had the highest averages: *Pneumonia* (24.9%), bacteremia (20.2%), surgical site (19.7%) and urinary tract (10.7%). However, they also varied between them, as can be seen in Table 1: Surgical site NIs decreased from 25.5% to 13% in 2020 and fell from first to fourth place. Bacteremia also decreased from 16.3% to 15%, after a high of 24.4% in 2018; moving from second to third place. Urinary tract infections more than doubled their contribution during the period from 8% to 17.1%, rising to third place. And pneumonias increased 2.4 times (from 14.8% to 35.5%), so it went from third to first place.

In regards to their causative agents, as shown in (Figure 1), during this six-year period, despite remaining in the two pranks, the five main bacteria have varied their influence on the etiology of NIs. Their combined frequency decreased from 60.3% to 59% of the total, with different particular changes. *E. coli* dropped from first place from the fourth year examined, placing third in 2020. *S. aureus* and *K. pneumoniae* eventually retained their second and fifth sites, respectively *P. aeruginosa* dropped from third to fourth place. And *A. baumannii* raised its percentage 2.2 fold, since the fourth year of observation, reaching a peak in 2018 with almost a fifth (18%) of all the NIs.

Figure 2 shows the rates of health care-associated infections (per 100 NI) of its five main causative agents from 2014 to 2020. On average, they showed an upward trend in their combined morbidity rate, increasing 2.7 times the value of 2014 (3.4%) in 2019 (9.2%), also with particular variations of each causal agent. Although all of them increased their hospital morbidity rates during this period, while *E. coli* and *P. aeruginosa* increased theirs by just over a point, *S. aureus* and *K. Pneumoniae* increased theirs up to 2.7 times. And *A. baumann*nii augmented 4.5 times its initial morbidity rate. In this period there were 8 hospital outbreaks (2 on average each year) due to pneumonia usually associated with mechanical ventilation; 5 caused by *A. baumannii* and 3 by *S. aureus*. As noted, in 2020 the frequency of detection of the five main causal agents coincides with the rates of their NI etiology.

The risk of these five bacteria causing nosocomial infections is much higher than the remaining 39 that were identified in 2020 (OR=16.5 and p=0.000). But differentially according to the infection: Much higher in those of surgical site (OR=12.4, p=0.000), intermediate in bacteremia (OR=2.59, p=0.000) and lower in pneumonia (OR=1.72, p=0.000) and urinary tract (OR=1.29, but p=0.17). And also differential for each causal agent, presenting a higher risk than its competitors in cases of *E. coli* urinary tract infection (OR=5.48, p=0.000); *A. baumannii pneumonia* (OR=2.81, p=0.000); *K. pneumoniae bacteremia* (OR=1.9, but p=0.12): and *S. aureus* surgical site, but not significantly (OR=0.96 and p=0.87). As shown in Table 2, during the period 2014-2020 global antimicrobial resistance of such bacteria showed ups and downs; while raised by *P. aeruginosa* (+13.2%) and *A. baumannii* (+8.6%), *K. pneumoniae* did not vary significantly (–0.7%), and *E. coli* (–20.7%) declined and much more *S. aureus* (–36.4%). And also, with differences according to the type of NI: Although *E. coli* and *S. aureus* presented decreases in their overall resistance in all NIs and, on the contrary, *A. baumannii* increased them in all; *P. aeruginosa* increased it in urinary tract infections and even more in bacteremia, decreasing it in pneumonia and surgical site; and on the other hand, in *K. pneumoniae* it grew in pneumonia, urinary tract and surgical site, declining in bacteremia. *A. baumannii* increased its resistance to almost all antimicrobials and

Table 1: Percentage distribution of major nosocomial infections in HGR 1, 2014-2020.

Year	Surgical Site	Bacteriemia	Pneumonia	Urinary Tract	Remainder
2014	25.5	16.3	14.8	8	35.4
2015	21.9	17.5	16.4	8.2	36
2016	22.8	21.7	21.2	6.8	27.5
2017	20.1	22.3	31.7	11.1	14.8
2018	16.6	24.4	27.3	13.2	18.5
2019	18.2	24	27.2	10.9	19.7
2020	13	15	35.5	17.1	19.4
Mean	19.7	20.2	24.9	10.7	24.5

Table 2: Percentages of global antimicrobial resistance of the main causative agents of main nosocomial infections. HGR 1, 2014 and 2020.

Agents	Pneumonia		Urinary tract		Surgical site		Bacteremia		Total Mean	
Causal	2014	2020	2014	2020	2014	2020	2014	2020	2014	2020
A. baumannii	81.1	82.6	82.9	89.1	79.2	88.1	78.7	87.2	78.8	87.4
E. coli	54.5	50.4	48.2	47.1	70.2	52.8	56.9	46.5	57.4	36.7
P. aeruginosa	60.7	54.8	60.5	74.4	73.8	70.9	55.5	95.4	62.6	75.8
K. pneumoniae	39	40.3	59.5	62.2	59.6	63.3	40	34	52.7	52
S. aureus	61.3	18.1	37.2	0	66.9	28.5	67.6	20.1	58.2	21.8

Table 3: Percentages of resistance to the classes of antimicrobials (according to the target of action) of the main causative agents of nosocomial infection. HGR 1, 2014 and 2020.

Agents	Cell wall synth.		Protein synth.		Nucleic acid synth.		Folates biosynth.		Total	
Causal	2014	2020	2014	2020	2014	2020	2014	2020	2014	2020
A. baumannii	66.7	87.7	80	79.7	73.3	100	88.9	100	70.5	87.4
E. coli	72	53.7	48.7	17.9	80.8	75	70.1	67.9	66.6	36.7
P. aeruginosa	80.2	75.6	64.4	47	72.2	58.8	75	100	75.4	75.8
K. pneumoniae	31.1	55.6	35	25	35	43.7	57.1	87.5	33.1	52
S. aureus	58.2	3.9	30.4	18.3	69.6	66.7	0	0	51	21.8

is practically only sensitive to Colistin (94.3%) and Tigecycline, but decreased its resistance to the latter derivative of tetracyclines up to 46.3% with high statistical significance (OR=2.67 and p=0.028). *P. aeruginosa*, despite some declines, also increased its overall resistance very significantly (OR=9.6 and p=0.000); the same as *K. pneumoniae*, although to a lesser degree (OR=1.7 and p=0.000). On the contrary, the decrease in global antimicrobial resistance of *S. aureus* (OR=2.13 and p=0.000) was also significant, and even more so in the case of *E. coli* (O =3.43 and p=0.000). But the changes were differential for each class of antimicrobial, according to their target of action. As shown in Table 3, although the percentages of global antimicrobial resistance of the five bacteria studied increased by an average of 2.4%; this occurred mainly against folate biosynthesis inhibitors (Trimetroprim-Sulfametoxasol: 12.9%) and, to a lesser extent, to the neutralizers of nucleic acid synthesis (Fluoroquinolones: 2.7%). And on the contrary, they decreased their resistance against inhibitors of protein synthesis (aminoglycosides, tetracyclines and oxazolidonones: –14.1%) and cell wall (lactam and non-lactam β: –6.3%). However, with particular differences between the causal agents: Percentages of overall resistance decreased in this period for *E. coli*, *S. aureus* and *P. aeruginosa*. *E. coli* in all types, but differentially: from –2.2% (folate synthesis), to –30.8 (protein synthesis). From 0% (folate biosynthesis) to –54.3% (cell wall synthesis) and *P. aeruginosa* from –4.6% (cell wall synthesis) to –17.4% (protein synthesis), although, on the contrary, it increased by 25% its resistance to antibiotics that inhibit folate synthesis up to 100%. Conversely, those which increased their overall resistance to the antimicrobials were *K. pneumoniae* (+30.2%) and *A. baumannii* (+17.6%). The former, although it decreased against antibiotics that inhibit protein synthesis (by –10%), increased it for the other three types: from 8.7% (nucleic acid synthesis) to 30.4% (folate biosynthesis). *A. Baumannii*, on the other hand, also decreased its resistance to inhibitors of protein synthesis but did not significantly (by –0.3%); increasing it for the other three types: from 11.1% (folate biosynthesis) to 26.7% (nucleic acid synthesis), reaching 100% resistance against these and antibiotics that inhibit folate biosynthesis. With such itinerary during the period 2014-2020, consider in gall nosocomial infections together caused by each of the five main bacteria studied, they presented in 2020 the antimicrobial resistances shown in Table 4. The four Gram-negatives outperformed the Gram-positive in terms of global resistance: from 1.7 times (*E. coli*) to the quadruple (*A. baumannii*) of the global resistance of *S. aureus A. baumannii* had the highest antimicrobial resistance (87.4%), mainly against Fluoroquinolones (96.4%) and β-lactamics (96.3%) and minimal against Colistine (5.7%) and Tigecycline (46.3%). It was followed by *P. aeruginosa* (75.8% overall), especially with a 100% efficacy against Amoxicillin, Cephalotin, Cefuroxime, Cefotaxime and Tigecycline; and its minimum resistance was against Colistine (57.1%) and Gentamicin (54.8%). In third place, *K. pneumoniae*, with 52% overall resistance, achieved maximum values against Ampicillin (91.2%) and Cefuroxime (70.6%) and lowermost for Carbapenems (28.4%), Norfloxacin (35.3%) and Gentamicin (38.2%). And *E. coli*, lowest percentage of overall resistance among Gram-negatives (36.7%), mainly because it is zero against Ertapenem and Phospholicin, very low for Meropenem (1.3%) and Nitrofurantoin (4%); reaching its maximum against Ampicillin (60.5%). For its part, *S. aureus* had the lower percentage of global resistance to antimicrobials, mainly because it was null against Vancomycin, Tigecycline, Linezolid and Daptomycin; with minimal values for Tetracyclines (0.9%), Gentamicin and Trimetroprim-Sulfamethoxosol (1.3% both); and because its highest resistances were 55% (Oxacillin and Erythromycin).

Discussion

The general incidence of NIs in HGR 1 during the six-year period studied showed slight variations. In 2014 its rate was 8 per 100 hospital discharges and, with slight ups and downs (mean of 7.8 and standard deviation of 0.2), reached 8.1 in 2020. To this it must have contributed, for its part, that the HGR 1 operate throughout the period the Institutional program to prevention and control of the infections associated with healthcare settings 3; by means of 17 priority care processes monitored monthly: Epidemiological surveillance, hands hygiene, mechanic ventilation, inhalation therapy, central venous catheter, surgical procedures, intensive care, antiseptics and sterilization, isolation precautions, order, cleaning and disinfection, safe water, infectious biological waste, hospital clothes and nutrition.

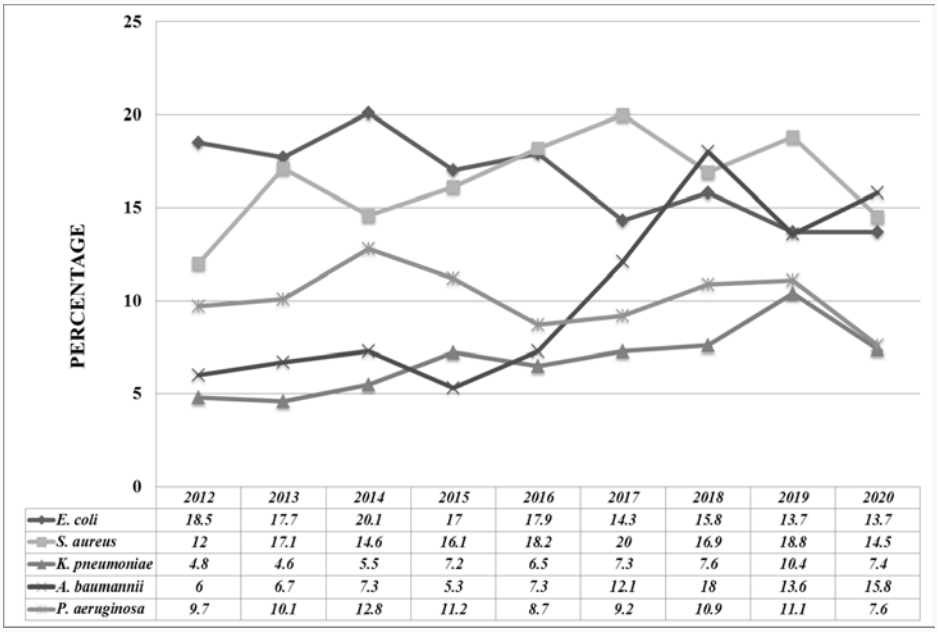
However, not only were the four main infections maintained, but

Table 4: Percentages of antimicrobial resistance of the main gram negative bacteria causing nosocomial infection. HGR 1, 2020.

TARGET	CLASIFICATION	CLASS	DRUG	*A. baumannil*	*E. coli*	*P. aeruginosd*	*K. pmeumoniae*
I ~ Cellwall Synthesis	ß Lactams	A-Pemicillias: Broad espectrum	Ampicillin		60.5		91.2
			Ampicillin/sulbactam	95	52.6		52.9
			Amoxicillia			100	
			Piperacilina			90.9	
			Ticarcillin			90.9	
		Sum		95	56.6	92	72.1
		B ~ Ceohalosporias:					
		1° Gemeration	Cephalotin	98.2	55.3	100	67.6
		2° Gemeration	Cefuroxime	98.7	52	100	70.6
		3° Gemeration	Cefotaxime	93.1	52	100	64.7
			Ceftaridime	93.2	52	61.3	61.8
			Ceftriaxome	97.4	53.9		64.7
		4° Gemeration	Cefepime	94.9	51.3	63.9	61.8
		Sum		96.1	52.8	84.4	65.2
		C ~ Carbapenems	Ertapenem		0		24.2
			Meropemem		1.3	68.6	32.3
		Sum			0.7	68.6	28.4
		D ~ Momobactams	Artreomam	100			
	Sum			96.3	43.1	82.7	59.3
	No lactams	Fosfomycia			0		
		Nitrofurantoin			4		45.4
		Colistin		5.7		57.1	
SUBTOTAL				87.9	36.3	81.2	58.1
II ~ Protein synthesis	1~Aminoglycosides		Amikacin		15.8	61.3	0
			Gentamycin	87.9	15.8	54.8	38.2
		Sum			15.8		19.1
	2- Tetracyclias		Tetracyclin	98			
			Minocycline	94.1			
			Tigecyclia	46.3		100	
SUBTOTAL				80.7	15.8	65.3	19.1
III ~ DNA	Flaorquinolomes	2° Gemeration	Ciprofloxacia	94.9	56.6	64.5	61.8
synthesis (DNA			Nor floracia		52.6	70.4	35.3
Topoisomerases)		3° Gemeration	Levofloxacia	98.1		77.8	
SUBTOTAL				96.4	54.6	69.7	48.5
IV - Folic acid	Sulfonamides	Trimethroprim	Trimethroprim/ Sulfamethoxazole	94.8	48		58.8
synthesis		Sulfamethoxazole					
TOTAL				87.4	36.7	75.8	52

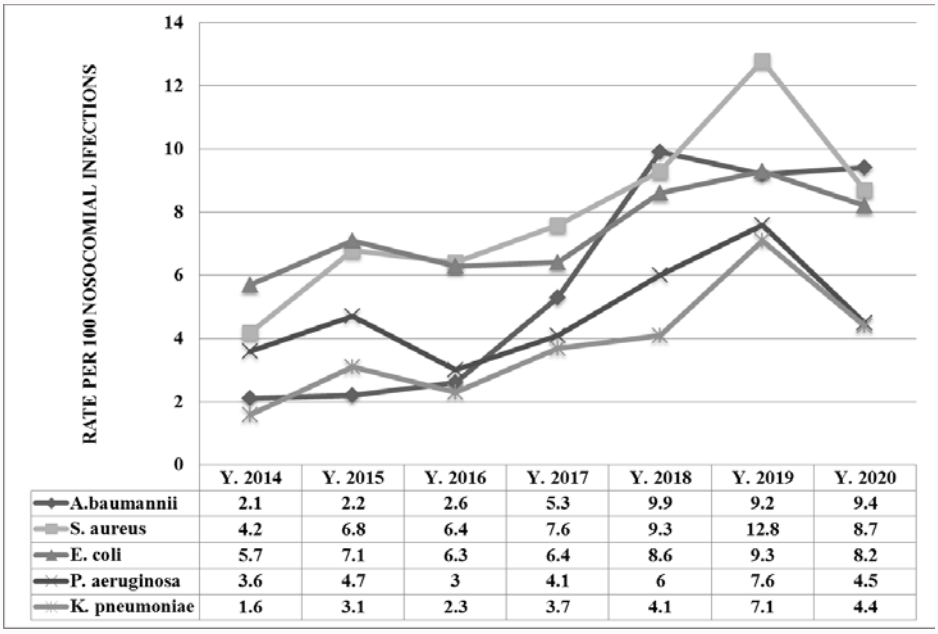
their total share was increased 14.7% (which implies an increase in the virulence and pathogenicity of their causative agents); through changes between them: Pneumonia and urinary tract ascending and bacteremia and surgical site decreasing. Likewise and also with variations, the majority of its five main causal agents remained, despite a slight decrease in the total (–1.3%); corresponding to the "superbugs "multidrug-resistant of the acronym ESKAPE (with *E. coli* instead of *Enterococcus faecium* and *Enterobacter* spp.) [6,7]. The changes in the incidence and importance of NIs are indicative of possible variations, both environmental (by de actions realized to prevention and control) and in the virulence and pathogenicity of the different strains and, therefore, of their genomes, even within the same species that cause them, since they persist.

Thanks to the plasticity of their genomes, bacteria can develop resistance to environmental stress caused by their physicochemical conditions, phages, detergents, antiseptics and antimicrobials; in order to evade them and adapt to the microenvironment, survive, reproduce and preserve the species. Thus, all mechanisms of antibiotic resistance are genetically developed and regulated, thanks to four fundamental processes: Gene mutation, activation of specific regulatory genes, global transcription tuning and horizontal transference. A classic example is the enzymes DNA gyrase and



	2012	2013	2014	2015	2016	2017	2018	2019	2020
E. coli	18.5	17.7	20.1	17	17.9	14.3	15.8	13.7	13.7
S. aureus	12	17.1	14.6	16.1	18.2	20	16.9	18.8	14.5
K. pneumoniae	4.8	4.6	5.5	7.2	6.5	7.3	7.6	10.4	7.4
A. baumannii	6	6.7	7.3	5.3	7.3	12.1	18	13.6	15.8
P. aeruginosa	9.7	10.1	12.8	11.2	8.7	9.2	10.9	11.1	7.6

Figure 1: Percentage distribution of main causative bacteria of nosocomial infections.



	Y. 2014	Y. 2015	Y. 2016	Y. 2017	Y. 2018	Y. 2019	Y. 2020
A.baumannii	2.1	2.2	2.6	5.3	9.9	9.2	9.4
S. aureus	4.2	6.8	6.4	7.6	9.3	12.8	8.7
E. coli	5.7	7.1	6.3	6.4	8.6	9.3	8.2
P. aeruginosa	3.6	4.7	3	4.1	6	7.6	4.5
K. pneumoniae	1.6	3.1	2.3	3.7	4.1	7.1	4.4

Figure 2: Nosocomial infection rates of the main causal agents.HGR 1, 2014-2020.

topoisomerase IV for DNA synthesis, through which damage by fluoroquinolones (Ciprofloxacin, Levofloxacin, Norfloxacin, etc.) is avoided due to mutations in DNA topoisomerases gyrA, gyrB and parC [25].

However, this process is contradictory, because although the "mutator" genes increase the mutation rate anywhere in the genome; the advantage of a mutating clone progressively deteriorates in a constant environment and the beneficial mutations decrease, increasing the harmful ones; thus becoming lethal. Thus, factors in the genetic system that increase variation can accelerate adaptive evolution; but genotypes that are already well adapted to a particular environment can also collapse. Thus, the evolution of genetic systems can represent a balance between these two opposing pressures [26].

Regarding the predominance of Gram-negative bacteria in the etiology of NI, it has been mentioned that it is due to their greater evolutionary development, which gives them more advantages, both in virulence and pathogenicity, as well as in their resistance to antimicrobials [25]. In principle, due to its particular cell wall or outer membrane, which constitutes a very efficient selective barrier for the regulation of the entry and ejection of substances from the external environment (such as β-lactam, glycopeptides, cephalosporins, carbapenems, antibiotics and others), through porin channels and efflux pumps; to which is compounded to a greater capacity to develop bio films and systems such as Quorum Sensing (QS) and chemical

signaling self-inducer for communication between bacteria; or as the indole produced by *E. coli* to modify cellular functions related to gene expression, mobility, adhesion and pathogenicity in response to environmental stress, including that caused by antimicrobials. Even among some shared mechanisms of intracellular resistance Gram-negatives surpass Gram-positives, such as *S. aureus*; such as oxide-reduction potential, inactivating enzymes of the transfer group or transferase (which neutralize aminoglycosides, chloramphenicol, macrolides and others), ribosomal control proteins (RPPs), etc. This is due to the greater number of specific genes regulating them in Gram-negatives (e.g., tetM, O, S, W, Q, T, A and B); compared to Gram-positive (tetM and O) [25].

Although global antimicrobial resistance increased in the five NI studied, it was contradictory. While *A. baumannii* achieved it in all NI, *E. coli* and *S. aureus* decreased it in all five. *P. aeruginosa* increased it in cases of urinary tract and bacteremia; decreasing it, on the contrary, in pneumonia and surgical site. In turn, *K. pneumoniae* also increased its resistance in urinary tract, pneumonia and surgical site; decreasing it in bacteremia. Then, such resistance impacted, together with the development of virulence and pathogenicity, on the modifications of the epidemiological profile of NI in HGR 1. Such increases with the irradiations in the morbidity of NI imply changes in the virulence and pathogenicity factors of their causative agents and, necessarily, of their genomes; even between different strains of the same species, while colonizing and infecting different tissues, organs and physiology, require, in first instance, changing capacities for insertion, adaptation (fitness), survival and reproduction indifferent environmental micro environments and, in the second instance, the development of different "armaments" or defensive mechanisms, mainly against antimicrobials.

As has been observed in the case of Methicillin-resistant *S. aureus*, on the one hand, "the susceptibility of hosts, sick or immunocompromised individuals, plays the main role in the emergence of new strains... resistant to antibiotics... by selective pressure in the field of health care"; and on the other hand, this is the result of "rapid bacterial adaptation and evolution, involving the development of resistance to antibiotics and the increase in their virulence and/or transmissibility" [27]; for which "the fluidity of intragenomic modifications is the main process of diversification and evolution of the genomes of pathogenic bacteria". It has also been shown in the case of *P. Aeruginosa* that "the evolution of the genome is mainly due to the acquisition of mobile genetic elements, gain and loss of genes, recombination events and extensive modifications"; this being "the main and/or the fastest mode of evolution of this bacterium" [28].

The variations in the global resistances presented by the bacteria studied were differential to the four classes of antimicrobials, while they do not show the same capacity against all. Where a sit increased for inhibitors of folate and nucleic acid synthesis, it also decreased against neutralizers of protein synthesis and cell wall. Thus, although in *S. aureus* all were decreased, it did much more (–54.3%) against inhibitors of cell wall synthesis (β-lactam and non-lactam), similar to *P. aeruginosa*, but to a lesser degree (–4.6%). While *E. coli*, which also decreased its resistance to all four classes, did so mainly (–30.8%) against those which inhibit protein synthesis (aminoglycosides and tetracyclines); such as *K. pneumoniae* which only decreased its resistance to this class of antimicrobials by –10%. Only *A. baumannii* increased its resistance to all four classes, despite the specific decrease against Tigecycline.

According to what has been proven, "the integration of exogenous genes is the most efficient of the means by which bacterial species can survive various environmental challenges, including exposure to antimicrobial compounds... The incorporation of Integrons provides the recipient bacterium with new proteins and new enzymatic functions, giving it a potential advantage for its evolutionary adaptation" [29].

Examples of their genomic variability for the development of their ability to inhibit antimicrobials are for *A. baumannii*: "Ambler B class is an acquired resistance mechanism that is located on the chromosome or in plasmids that hydrolyze all β-lactam antibiotics, except Aztreonam" [30]; Metallo-B-Lactamases (MBL's) are encoded by plasmids (against Penicillins, first through fourth generation Cephalosporins, Carbapenems, etc.); and "since MBL's are generally located in mobile genetic elements, they are easily transmitted between bacteria" [31]. Changes in outer membrane proteins "are generally associated with porin mutation with overproduction of cyclic adenosine monophosphate (AmpC)". And specifically, resistance to Carbapenems "is mediated by the conversion of the OXA-66 gene into OXA-82 and its subsequent overexpression". For its part, the efflux pump rests "on the ade-R or ade-S gene and a single point mutation produces an expanded expression and, with it, a greater effluence "of harmful substances [32]. But any this does not take place in a linear course, but with ups and downs and contradictorily.

Thus, for example, the accelerated overtake of *A. baumannii* over its competing microorganisms (rising from fifth place in frequency to the first in three years, from 2015 to 2018) and its great increase in the etiology of nosocomial infections (increasing its NI rate by 4.5 times in that triennium) constitute a trend; and, not equitably, its preponderance increased somewhat in 2016, to fall down to third place in 2019 and retake the first place in 2020; and while its incidence in pneumonia and bacteremia increased, it decreased in urinary tract infections and surgical site. And although it increased its overall resistance to antimicrobials, it did not do so to the same extent against everyone (more for inhibitors of nucleic acid– fluoroquinolones– and cell wall – β-lactam – and less for those that inhibit folate synthesis: Sulfonamides) and in the case of those that inhibit protein synthesis (aminoglycosides and tetracyclines) it even decreased its resistance a little although not significantly. Such variations are likely to be due, in turn, to variant strains for the development of different pathogenic capacities of each species for the colonization and infection of different tissues, organs, apparatuses and host systems.

E. coli, for example, originally adapted to the intestinal tract, had to evolve into variant strains in order to cause infections in the urinary tract, lungs, surgical wounds and blood; giving rise to two groups of strains that infect humans: diarrheagenic and extra intestinal, such as O157:H7 capable of successfully developing in various environmental microenvironments [33]. Thus, in order to infect the urinary tract from intestinal colonization it requires mainly morphological developments (fimbria, pili, capsules, outer membrane proteins, etc.), mobility (flagella) and adhesion (adhesins) or chemotaxis, the production of various O antigens and toxins (such as α-hemolysin, necrotizing cytotoxic factor-1 and protease related to colonization) and, therefore, specific genomic changes [34]. And likewise, of different defense weapons, even against the same antimicrobials due to their different pharmacodynamics and pharmacokinetics.

This process of adaptation is determined by genomic antecedents and their evolution, due to three basic effects: The possession of functions already in disuse (for survival in a past environment and mechanisms of resistance to absent antibiotics); variation in activities to meet vital nutritional needs (such as siderophores for iron uptake, which "are clearly required for successful infection, in addition to zinc and manganese") and metabolic (amino acids and peptides as primary carbon resources, critical for adaptation to the microenvironment– fitness– and growth); and evasion of host defense mechanisms (specific antigens) [35].

Instead, several factors explain the predominance of *A. baumannii*: in principle, its "great adaptability to adverse environmental conditions, due to its ability to persist for many days in dry and rigorous environments, such as those in hospitals" [36], thanks to the elaboration with biofilm of "a colonial niche whose contact with humans results in its infection. The hydrophobic polysaccharide and pili surface of the bacterial cell surface initiates adhesion to human epithelial cells, thus initiating the infectious process" [37]. And as has been observed, "the increase in the selection of survivors to harmful environmental factors, such as clinical disinfectant strategies, antimicrobial compounds, etc., select strains that manifest a hyper virulent phenotype such as the multidrug-resistant to antibiotics" [38]; being five the main factors of its virulence: hydrophobicity and cell surface enzyme, secretion of toxic polysaccharides for neutrophils (D-glucoronic acid, D-mannose, L-ramnose and D-glucose), antigenic verotoxins (vtx-1 and vtx-2), siderophores and external membrane proteins (as in other Gram-negative) [36].

As for their mechanisms of antimicrobial resistance, the main ones are: Inactivating enzymes such as β-lactamases, changes in external membrane proteins (against penicillins and carbapenems), efflux pumps ("responsible for resistance to aminoglycosides, quinolones, tetracyclines, chloramphenicol, erythromycin, trimethoprim and ethidium bromide"), enzymatic inactivation of the antimicrobial (N-acetyl transferases, acetylating and phosphorylating enzymes, etc. anti-aminoglycosides) and mutation of DNA polymerases (DNA gyrase gyr-A and Topoisomerase IV par-C inhibitors of fluoroquinolones) [29]. And specifically: their resistance requires "the presence of genes encoding aminoglycoside-modifying enzymes within class 1 of integrons the genes responsible are usually concealed in transposons within transferable plasmids, allowing their horizontal transmission". The "alteration in the structure of DNA gyrase or topoisomerase IV by mutations, determinant of regions of the gyrA and parC genes, is the main cause of resistance of *A. baumannii*" to these antibiotics. Two types of resistance to tetracyclines and glycylcyclines have been described: "TetA and TetB are specific transposon mediators of effluent pumps"; and the ribosomal protective protein, which protects it from the former, and which is encoded by the tet(M) gene; and Tigecycline "is also a substrate for the emerging effluence system" [29].

And in the case of the Gram-positive bacterium, *S. aureus*, maintained throughout the period studied a continuous battle for preponderance against Gram-negatives, although it returned to second place in frequency in 2020, after occupying the first in 2017, it also achieved the first place in the etiology of all NI in 2019. This notwithstanding the biological and evolutionary limitations it has with respect to *A. baumannii* and *E. coli*, for example. For although it shares with them the ability to produce biofilms (even in their co-habitation with them), transferases (to neutralize aminoglycosides, chloramphenicol, macrolides and others) and that, through the PABA (Para-Amino-Benzoic Acid) precursor of bacterial folic acid, can cancel out the effect of sulfonamides [25] and, even, its great success against Methicillin [39]; it has fewer specific genes for all this than its competitors [25]. Thus, it surprises then its decrease in global resistance to antimicrobials, reaching zero percent in urinary tract infections, also zero against Vancomycin, Tigecycline, Linezolid and Daptomycin, and minimal (1.3%) for Gentamicin, Tetracycline, Doxycycline and Trimethoprim-Sulfamethoxazole. In this case, its virulence factors and its pathogenicity potential must exceed its antimicrobial resistance capacity.

As has been shown in various ways, the evolution of the causative agents of NI in terms of their virulence, pathogenicity and antimicrobial resistance is not a linear and always ascending process; but on the contrary, with ups and downs, contradictions and regressions [40]. This is due, as Richard Lenski has explained, to the fact that "periods of constant increase are interrupted, however, by sudden decreases in the frequency of [genetic] mutations. These decreases result from the selection of favorable mutations in other locuses, which tend to occur in the population portion of the [original] numerically dominant strains" [11].

Conclusion

Although the incidence of nosocomial infections is a global process, it takes place due to particular variations in time and in its local properties in each region, country and even in each hospital [41]. In the studied case, for example, although ESKAPE bacteria also predominate as in the globe, instead of *Enterococcus* or *Enterobacter*, which is common, *Escherichia coli* occupied its place. And there is a fierce competition between them for transmission, contagion, colonization and etiology of infections. And according to Charles Darwin, "as many more individuals of each species are born than can possibly survive; and as, consequently, there is a frequently recurring struggle for existence, it follows that any being, if it vary however slightly in any manner profitable itself, under the complex and sometimes varying conditions of life, will have a better chance of surviving, and thus be naturally selected. From the strong principle of inheritance, any selected variety [strain in this case] will tend to propagate its new and modified form" [10]. This is clearly seen in the case of *A. baumannii* that in just three years overcame all competition and obstacle, placing itself in the first place, colonizing and infecting other tissues, organs, apparatuses and systems, other than the primary one [42]. Such development can only occur thanks to bacterial improvements of three interrelated fundamental factors: Virulence, pathogenicity and resistance to hygiene actions and the intensive use of antiseptics and antimicrobials in the hospital. Already with very limited sensitivity to antimicrobials, other weapons against this bacterium are being investigated, including bacteriophages instead of antibiotics [43,44].

Consequently, only through the theory of evolution by natural selection, applied to the causal agents of the health care associated infections, it is possible to understand and explain its process and specify the most appropriate strategies for its prevention, control and abatement. This is particularly required for the definition of recommendations for a rational use of antimicrobials over a specific period of time and for a specific hospital.

Thus, it was possible to determine for HGR 1 in the year 2020 which antibiotics are most likely to be effective (<20% to 25%

resistance [45], in the cases of its main and majority nosocomial infections, according to their causal agents. In general, against *A. baumannii*: Colistin and Tigecycline; *S. aureus*: Vancomycin, Tigecycline, Linezolid, Daptomycin, Doxiciclin and Trimethoprim-Sulfamethoxazole; *E. coli*: Ertapenem, Fosfomycin, Meropenem and Nitrofurantoin; *P. aeruginosa*: Gentamicin, Colistin, Amikacin and Ceftacidim; *K. pneumoniae*: Amikacin, Ertapenem, Meropenem and Norfloxacin; etc.

Consequently, it is necessary to integrate a perspective of evolution by natural selection of the causative agents of nosocomial infections in their epidemiological active surveillance, based on their daily study with cultures and antibiogram and, whenever possible, the determinants genetic studies; in order to detect in a timely manner the changes in the pathogenic hospital microbiome and its sensitivity and resistance to antimicrobials and, with this evidence, formulate, specify, perform, evaluate and feedback the most effective strategies for the control, therapeutics and abatement of the incidence of the health care associated infections. This is the best way to contribute to the WHO Global Strategy to Contain Antimicrobial Resistance [46], through the Global Antimicrobial Resistance Surveillance System [47], and also to the implementation of the Global Action Plan on Antimicrobial Resistance formulated by the World Health Organization [48].

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References

1. Lax S, Gilbert JA. Hospital-associated microbiota and implications for nosocomial infections. Trends Mol Med. 2015;21(7):427-32.
2. Westwood J, Burnett M, Spratt D, Ball M, Wilson D, Wellsteed S, et al. The hospital microbiome project: Meeting report for the UK science and innovation network UK-USA workshop "beating the superbugs: Hospital microbiome studies for tackling antimicrobial resistance", October 14th, 2013. Stand Genomic Sci. 2014;9:12.
3. IMSS (Instituto Mexicano del Seguro Social): Programa institucional de prevención y control de infecciones asociadas a la atención de la salud 2019-2024. Ciudad de México, 2019.
4. McFall-Ngai M. Adaptative immunity: Care for the community. Nature. 2007;445(7124):153.
5. Ed Yong. Yo contengo multitudes. Los microbios que nos habitan y una visión más amplia de la vida. SAU España: Penguin Random House Group Editorial; 2017.
6. Rice LB. Federal funding for the study of microbial resistance in nosocomial pathogens: No ESKAPE. J Infect Dis. 2008;197(8):1079-81.
7. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int. 2016;2016:2475067.
8. Wu HJ, Wang AHJ, Jennings MP. Discovery virulence factors of pathogenic bacteria. Curr Opin Chem Biol. 2008;12(1):93-101.
9. Stephen JG, Lewontin RC. Biological adaptation. Scientific World. 22(3):214-22.
10. Charles D. The origin of species by means of natural selection, or the preservation of favored races in the struggle for life. New York: Appleton and Company; 1861.
11. Richard EL. Evolution in action: at 50,000 - Generation salute to Charles Darwin. Microbe. 2011;6:1.
12. Junquera S, Loza E, Baquero F. Evolution of the sensitivity pattern of Escherichia coli isolates in urine cultures from the hospital and extra hospital environment. Enferm Infect Microbiol Clin. 2005;23(4):197-201.
13. Lewontin RC. Genes, organismo y ambiente. Las relaciones de causa y efecto en biología. Barcelona: GEDISA; 2000.
14. Dawkins R. El relojero ciego. SA Barcelona; RBA Editors; 1993.
15. World Health Organization. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. Geneve: Switzerland; 2017.
16. Salazar-Holguin HD, Salazar-Fernandez EP. Bacterial evolution of urinary tract infections acquired in the community and in the hospital. A case of Mexico. Health Prim Car. 2019;2(4):1044.
17. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74(3):417-33.
18. OMS: Sistema mundial de vigilancia de la resistencia a los antimicrobianos. Manual para la primera fase de implementación. Organización Mundial de la Salud. Ginebra; 2017.
19. Nester-Eugene W. Mecanismos de acción de los antibacterianos; en: Microbiología humana. México: Manual Moderno; 2009. p. 550-7.
20. Molina-López J. Drogas anti bacterianas. Facultad de Medicina de la UNAM. México: DF Agosto; 2012.
21. Nester-Eugene W. Resistencia a los antimicrobianos; en: Microbiología humana. México: Manual Moderno; 2009. p. 561-4.
22. Vignoli R, Seija V. Principales mecanismos de resistencia antibiótica; en: Temas de bacteriología y virología médicas. Facultad de Medicina de la Universidad de la República, 3rd Ed. Montevideo; 2008.
23. IMSS (Instituto Mexicano del Seguro Social). Manual de toma, manejo y envío de muestras biológicas para la Vigilancia Epidemiológica basada en Laboratorio. Ciudad de México; 2017.
24. Salazar-Holguín HD, Cisneros-Robledo ME. Resistencia a los antimicrobianos de agentes causales de las principales infecciones nosocomiales. Rev Med Inst Mex Seguro Soc. 2016;54(4):190-5.
25. Troncoso C, Pavez M, Santos A, Salazar R, Barrientos L. Structural and pshysiological implications of bacetrial cell in antibiotic resistance mechanisms. Int J Morphol. 2017;35(4):1214-23.
26. Molina-López J, Aparicio-Ozores G, Ribas-Aparicio RM, Gavilanes-Parra S, Chavez-Berrrocal ME, Hernandez-Castro R, et al. Drug resistance, serotypes and phylogenetic groups among uropathogenic Escherichia coli including O25-ST 131 in Mexico City. J Infect Dev Ctries. 2011;5(12):840-9.
27. Spicknall IH, Foxman B, Marrs CF, Eisenberg JNS. A modeling framework for the evolution and spread of antibiotic resistance: Literature review and model categorization. Am J Epidemiol. 2013;178(4):508-20.
28. Olaechea PM, Insausti J, Blanco A, Luque P. Epidemiología e impacto de las infecciones nosocomiales. Med Intensiva. 2010;34(4):256-67.
29. Mazel D. Integrons: Agents of bacterial evolution. Nature. 2006;4:608-20.
30. Almasaudi SB. Acinetobacterspp as nosocomial pathogens: Epidemiology and resistance features. Saudi J Biol Sci. 2018;25(3):586-96.
31. Alexis DP. Infecciones por Acinetobacter baumannii pan-resistente. Consideraciones epidemiológicas y de manejo antimicrobiano actualizado. Rev Chil Infect. 2005;22(4):298-320.
32. Zander E, Chmielarczyk A, Heczko P, Seifert H, Higgins PG. Conversion of OXA-66 into OXA-82 in clinical *Acinetobacter baumannii* isolates and association with altered carbapenem susceptibility. J Antimicrob Chemother. 2013;68(2):308-11.

33. Souza V, Castillo A, Eguiarte LE. Evolutionary ecology of *Escherichia coli*. Caracas. 2001.

34. Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic Escherichia coli (UPEC) infections: Virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Front Microbiol. 2017;8:1566.

35. Derakhshandeh A, Firouzi R, Motamedifar M, Arabshahi S, Novinrooz A, Boroojeni AM, et al. Virulence characteristics and antibiotic resistance patterns among various phylogenetic groups of uropathogenic Escherichia coli isolates. Jpn J Infect Dis. 2015;68(5):428-31.

36. Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of *Acinetobacter* spp: An overview. Microbes Environ. 2011;26(2):101-12.

37. Gebhardt MJ, Gallagher LA, Jacobson RK, Usacheva EA, Peterson LR, Zurawski DV, et al. Joint transcriptional control of virulence and resistance to antibiotic and environmental stress in *Acinetobacter baumannii*. mBio. 2015;6(6):e01660-15.

38. Gallego L. *Acinetobacter baumannii*: Factors involved in its high adaptability to adverse environmental conditions. J Microbiol Exp. 2016;3(2):00085.

39. Uhlemann AC, Otto M, Lowy FD, DeLeo FR. Evolution of community and health care associated methicillin-resistant *Staphylococcus aureus*. Infect Genet Evol. 2014;21:563-74.

40. Aujoulat F, Roger F, Bourdier A, Lotthe A, Lamy B, Marchandin H, et al. From environment to man: Genome evolution and adaptation of human opportunistic bacterial pathogens. Genes (Basel). 2012;3(2):191-232.

41. WHO (World Health Organization): Worldwide country situation analysis: Response to antimicrobial resistance. Geneva; 2015.

42. Salazar-Holguin H. *Acinetobacter baumannii* antibiotic multiresistance evolution in hospital acquired infections, clinical data from a six year study in Mexico. Am J Clin Microbiol Antimicrobial. 2018;1(5):1023.

43. Knox A. Phage therapy is effective against the superbug *Acinetobacter baumannii*. Massive Science. 2021.

44. Altamirano FG, Forsyth JH, Patwa R, Kostoulias X, Trim M, Sudedi D, et al. Bacteriophage-resistant *Acinetobacter baumannii* are resensitized to Antimicrobials. Nat Microbiol. 2021;6(2):157-61.

45. Cárdenas-Perea M. Factors of bacterial virulence: The 'intelligence' of bacteria. Elements. 2014;9:35-43.

46. WHO. Estrategia mundial de la OMS para contener la resistencia a los antimicrobianos. Organización Mundial de la Salud. Geneva; 2001.

47. OPS. Sistema mundial de vigilancia de la resistencia a los antimicrobianos. Manual para la primera fase de implementación. Organización Mundial de la Salud. Ginebra; 2017.

48. WHO. Global action plan on antimicrobial resistance. Geneva: Switzerland; 2015.