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Active surveillance of adult healthcare-associated infections in intensive care units: resistance and molecular profile in an upper middle-income country

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Abstract

Objective: This study aimed to characterize epidemiological and molecular profile of Healthcare-associated infections [HAI] in 21 intensive care units (ICU) in a city in Colombia.

Methods: Descriptive study of prevalence. Adult patients were screened in 21 ICUs for HAIs: VAP, CLABSI; CAUTI and/or SSI. Microbiological and genotypic identification was performed.

Results: Prevalence of HAIs was 41.4% (CI 36.9-45.9).VAP 15.8% (CI 12.7-19.4); CLABSI, 13.5% (CI 10.6-16.9); CAUTI, 7.7% (CI 5.5-10.5); and SSI, 4.4% (CI 2.7-6.6). Gram-negative bacteria (71.7%) predominated (*P. aeruginosa* (19.1%), *K. pneumoniae* (13.4%) and *E. coli* (13%)). *Pseudomonas* spp. 20-30% were resistant to carbapenems and ≥ 10% to aztreonam, 3rd- and 4th-generation cephalosporins, and β-lactamase inhibitors. In VAP and CLABSI, 30% of *Staphylococcus aureus* were resistant to oxacillin. In CAUTI, *Staphylococcus epidermidis* exhibited 100% resistance. In *P. aeruginosa* resistance gene were blaTEM, blaSHV, and blaCTX-M (15-32%), KPC (5.7%), and oxacillinases blaOXA-48 (1.8%) and blaOXA-1-40-30 (20-50%). In *E. coli*, genes qnrB, qnrS and qnrD were identified. In CLABSI, ermC-type (16.7%), aph[2']'If (7.7%) and ant[4']-Ia (7.7%) were identified in *Staphylococcus aureus*.

Conclusions: VAP and CLABSI predominate in ICUs evaluated in Colombia due to resistant gram-negative bacteria by ESBL-type resistance genes plasmids, efflux pumps hindering the therapeutic approach.

Keywords: Molecular Epidemiology; Infection Control; genotyping, Colombia.

Vigilancia activa de infecciones asociadas a la atención en salud en unidades de cuidado intensivo: perfil de resistencia y molecular en un pais en desarrollo

Resumen

Objetivo: Caracterizar el perfil epidemiologico molecular de infecciones asociadas al cuidado de la salud (IACS) en 21 unidades de cuidado intensivo (UCI) en una ciudad en Colombia.

Metodos: Estudio descriptivo de prevalencia. Pacientes adultos fueron tamizados en 21 UCI para IACS: VAP, CLABSI; CAUTI y/o SSI. Se hizo identificación microbiologica y de genotipos.

Resultados: La prevalencia of IACS fue de 41,4% (IC 36,9-45,9). VAP 15,8% (IC 12,7-19,4); CLABSI, 13,5% (IC 10,6-16,9); CAUTI, 7,7% (IC 5,5-10,5); y SSI, 4,4% (IC 2,7-6,6). Predominaron bacterias Gram-negativas con 71,7%: *P. aeruginosa* (19,1%), *K. pneumoniae* (13,4%) y E. coli (13%). *Pseudomonas* spp. en 20-30% fueron resistentes al carbapenem y ≥ 10% al aztreonam, cefalosporinas de 3ra- y 4a-generacióne inhibidores de β-lactamasa. En VAP y CLABSI, el 30% de Staphylococcus aureus fueron resistentes a oxacilina. En CAUTI, *Staphylococcus epidermidis* mostró 100% de resistencia. Los genes de resistencia en *P. aeruginosa* fueron blaTEM, blaSHV, y blaCTX-M (15-32%), KPC (5.7%), y oxacillinasas blaOXA-48 (1,8%) y blaOXA-1-40-30 (20-50%). In *E. coli*, los genes qnrB, qnrS y qnrD fueron identificados. En CLABSI, ermC-type (16,7%), aph[2']'If (7,7%) y ant[4']-Ia (7.7%) se identificaron en *Staphylococcus aureus*.

Conclusiones: VAP y CLABSI predominaron en la UCI en Colombia principlamente por presencia de bacterias gram-negativa con plasmidos tipo ESBL de bombas de flujo, dificultando el tratamiento de estos casos.

Palabras clave: Epidemiología Molecular; cuidados intensivos; Control de infecciones; genotipaje; Colombia.

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Introduction

The Surveillance of Healthcare-Associated Infections [HAI] in Infection Control guarantees safety in patient care. HAIs are defined as pathologies resulting from healthcare that are absent in hospital admission[1,2].

Epidemiology is complex, as there is heterogeneity in the quality of information and the knowledge gap in surveillance policy in low- and middle-income countries. Patients hospitalized in Intensive Care Units (ICUs) in developed countries are affected by at least one episode of HAI, with high incidence ranging from 5.7 to 19.1% with frequencies of 29% in Surgical Site Infections [SSIs] and 24% Urinary Tract Infections [UTIs] and 19% Bloodstream Infections [BSIs], 14.8% hospital pneumonia and the remaining 13.1% other infections[3].

The etiology is sometimes complex to establish; HAIs are reported predominantly in ICUs related to gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli,* and in gram-positive, coagulase-negative *Staphylococcus.* Regarding antimicrobial resistance, *Staphylococcus aureus* resistant to oxacillin (MRSA), resistance to 3rd generation cephalosporins in *E. coli* (16%), *Klebsiella spp*. (40%) and *Enterobacter spp*. (34%); and resistance to carbapenems such as *Klebsiella spp*. (15%), *P. aeruginosa* (26%) and *A. baumanii* (64%) predominate[4,5]. Resistance genes have been molecularly characterized, such as ones related to Extended-Spectrum ß-lactamases [ESBLs], Aminoglycoside Modifying Enzymes [AMEs], Plasmid-Mediated Quinolone Resistance [PMQR] or mutations in the *gyrA gene*[6,7].

The following study aimed to characterize the molecular profile of HAIs in 21 adult intensive care units in Cartagena de Indias, Colombia.

Material and methods

Study design

Research supported by the health authority of Cartagena in the case of a descriptive study of prevalence. Twenty-one adult care ICUs were monitored for: Ventilator-associated Pneumonia [VAP], Central Line-Associated Bloodstream Infection [CLABSI]; Catheter-Associated Urinary Tract Infection [CAUTI] and/or Surgical Site Infection [SSI]. Over 10 months, 481 adult patients with a diagnosis of HAI were included according to the criteria by the Centers for Disease Control and Prevention of the United States [CDC] and the National Institute of Health of Colombia [INS][8]. A biological sample was available for analysis. All patients and/or their families signed an informed consent to participate.

Phenotypic characterization and antimicrobial susceptibility profile

For phenotypic identification and susceptibility tests [sensitivity and minimum inhibitory concentrations-MIC-] the automated standardized method of the MicroScan4 analyzer [Beckman Coulter®] following CLSI [Clinical and Laboratory Standards Institute] principles were performed[9].

Genotypic characterization

The DNA was obtained using protocols standardized by the UNIMOL (Unidad Investigacion Molecular) laboratory and using the commercial kit for genomic DNA, *Wizard®* [Promega®] according to the manufacturer's recommendations. For molecular characterization, the endpoint Polymerase Chain Reaction [PCR] technique was used (see Table 1). According to the susceptibility profile, resistance genes against pharmacological groups of clinical interest were chosen. (Table 1) and sequenced using the Sanger technique and analyzed using BLAST online software[10].

Statistical analysis

The collected variables were analyzed according to their nature using descriptive statistics. SPSS IBM® was used for all analyses with a two-tailed significance level of 0.05.

Results

Prevalence of HAIs in Cartagena, Colombia

Over 10 months, 481 patients were screened, 282 were excluded (did not meet the inclusion criteria), the reasons for exclusion were not meeting the CDC criteria to define the IAAS cases in other cases information was incomplete; finally 199 patients were monitored, of which 90.5% (180) had a single HAI. A homogeneous distribution was observed in relation to sex (50.3% men). On average, the patients were 59.6 years old (SD 19.5 years old). The time elapsed between admission to the ICU and infection (HAI) in 50% of the population was 4 days (IQR 2-9), for CLABSI 50% of the patients had this infection at 5 days (IQR 2-13). Satisfactory recovery was observed in 78.3% of patients, and 12.6% died, with HAI being the final cause of death in 92% of cases. The event with the highest proportion of deaths was VAP (17.1%) (Table 2).

During the follow-up time, the prevalence of HAI was 41.4% (CI 36.9-45.9), VAP was the most prevalent at 15.8% (CI 12.7-19.4) followed by CLABSI 13.5% (CI 10.6-16.9), CAUTI 7.7% (CI 5.5-10.5) and SSI with 4.4% (CI 2.7 - 6.6) (Table 2).

Phenotypic characterization of microorganisms associated with HAI

Regarding the etiology, 14.7% of the cases of HAI presented polymicrobial etiology. In 9.4% of the samples, no germ was isolated. The distribution of microorganisms had a large predominance of gram-negative bacteria (71.7%), among which *Pseudomonas aeruginosa* (19.1%), *Klebsiella pneumoniae* (13.4%) and *Escherichia coli* (13%) were the most identified. Within the group of gram-positive (13.1%), *Staphylococcus aureus* (4.3%), *Staphylococcus epidermidis* (1.7%), and *Staphylococcus hominis* (1.7%) were the most identified. Regarding fungi (5.5%), *Candida famata* (2%) and *Candida albicans* (1.3%) were the most identified (Table 3). *Pseudomonas aeruginosa* was more frequent in VAP (24.3%), CLABSI (18%) and SSI (15.4%), while in CAUTI, *Escherichia coli* was found at 18.2% frequency.

Table 1. Resistance genes and primers used for the genotyping of microorganisms isolated in patients with HAI in Cartagena, Colombia.

GEN	SEQUENCES (5′ – 3′)	Primer size (bp) Annealing Temp. (Ta-°C)	Resistance/Reference Phenotype
ermA, ermC *(a)*	*ermA* F: AAG CGG TAA ACC CCT CTG A *ermA* R:TTC GCA AAT CCC TTC TCA AC *ermC* F: AAT CGT CAA TTC CTG CAT GT *ermC* R:TAA TCG TGG AAT ACG GGT TTG	*ermA* 190bp; Ta: 55 *ermC* 299bp; Ta: 55	Erythromycin -Clindamycin *ermA* [24] *ermC* [24]
mecA *(b)*	*mecA* F: AAA ATC GAT GGT AAA GGT TGG C *mecA R*: AGT TCT GCA GTA CCG GAT TTG C	*mecA* 532bp; Ta: 55	*ß-lactams* *mecA* [24]
TEM, SHV, KPC, CTX-M, MTSO, OXA 48, OXA 23 (c)	TEM F: GCG GAA CCC CTA TTT G TEM R: ACC AAT GCT TAA TCA GTG AG SHV F: TTA TCT CCC TGT TAG CCA C SHV R: GAT TTG CTG ATT TCG CTC GG KPC F: CGTCTAGTTCTGCTGTCTTG KPC R: CTTGTCATCCTTGTTAGGCGG CTX-M F: ATG TGC AGY ACC AGT AAR GTK ATG GC CTX-M R: TGG GTR AAR TAR GTS ACC AGA AYS AGC GG MultiTSO F: GGC ACC AGA TTC AAC TTT CAA G MultiTSO R: GAC CCC AAG TTT CCT GTA AGT G OXA 48 F: GCT TGA TCG CCC TCG ATT OXA 48 R: GAT TTG CTC CGT GGC CGA AA OXA 23 F: GAT CGG ATT GGA GAA CCA GA OXA 23 R: ATT TCT GAC CGC ATT TCC AT	TEM 1017bp; Ta: 55 SHV 795bp; Ta: 60 KPC 798bp; Ta: 55 CTX-M 593bp; Ta: 60 MultiTSO 564bp; Ta: 60 OXA 48 281bp; Ta: 60 OXA 23 64bp; Ta: 52	*ß-lactams* TEM[25] SHV [26] KPC [27] CTX-M [28] MTSO[29] OXA 48 [29] OXA 23 [30]
qnrA, qnrB, qnrS, qnrC, qnrD, aac(6′)-Ib-cr, qepA, oqxA, oqxB *(a and c)*	*qnrA* F: AGAGGATTTCTCACGCCAGG *qnrA R*: TGCCAGGCACAGATCTTGAC *qnrB* F: GGMATHGAAATTCGCCACTG *qnrB R*: TTTGCYGYYCGCCAGTCGAA *qnrS* F: GCAAGTTCATTGAACAGGGT *qnrS R*: TCTAAACCGTCGAGTTCGGCG *qnrC* F: GGG TTG TAC ATT TAT TGA ATC G *qnrC R*: CAC CTA CCC ATT TAT TTT CA *qnrD* F: CGA GAT CAA TTT ACG GGG AAT A *qnrD R*: AAC AAG CTG AAG CGC CTG *aac(6′)-Ib-cr* F: ATA TGC GGA TCC AAT GAG CAA CGC AAA AAC AAA GTT AG *aac(6′)-Ib-cr* R: ATA TGC GAA TTC TTA GGC ATC ACT GCG TGT TCG CTC *qepA* F: GCA GGT CCA GCA GCG GGT AG *qepA R*: CTT CCT GCC CGA GTA TCG TG *oqxA* F: CTCGGCGCGATGATGCT *oqxA* R: CCACTCTTCACGGGAGACGA *oqxB* F: TTC TCC CCC GGC GGG AAG TAC *oqxB* R: CTC GGC CAT TTT GGC GCG TA	*qnrA* 580bp; Ta: 54ºC *qnrB* 264bp; Ta: 54ºC *qnrS* 428bp; Ta: 54ºC *qnrC* 307bp; Ta: 55ºC *qnrD* 581bp; Ta: 57ºC *aac(6′)-Ib-cr* 519bp Ta: 50ºC *qepA* 199bp; Ta: 63ºC *oqxA* 392bp Ta: 68ºC *oqxB* 512bp Ta: 70ºC	Quinolones *qnrA* [31] *qnrB* [31] *qnrS* [31] *qnrC* [32] *qnrD* [33] *aac(6′)-Ib-cr* [32] *qepA* [34] *oqxA* [35] *oqxB* [35]
aac[3]-Ia, aac[6′]-Ib, aph[2″]If, aac[6′]-Ie/ aph[2″]Ia, ant[4′]-Ia *(a and c)*	*aac[3]-Ia* F: ATGGGCATCATTCGCACA *aac[3]-Ia* R: TCTCGGCTTGAACGAATTGT *aac[6′]-Ib* F: ATGACTGAGCATGACCTTG *aac[6′]-Ib* R: AAGGGTTAGGCAACACTG *aph[2″]If* F: AAGGAACTTTTTTAACACCAG *aph[2″]If R*: CCWATTTCTTCTTCACTATCTTC *aac[6′]-Ie/aph[2″]Ia* F: ACAGAGCCTTGGGAAGATGAAG *aac[6′]-Ie/aph[2″]Ia R*: TGTTCCTATTTCTTCTTCACTATC *ant[4′]-Ia* F: AATCGGTAGAAGCCCAA *ant[4′]-Ia R*: GCACCTGCCATTGCTA	*aac[3]-Ia* 484bp; Ta: 60ºC *aac[6′]-Ib* 524bp; Ta: 58ºC *aph[2″]If* 420bp; Ta: 50ºC *aac[6′]-Ie/aph[2″]Ia* 1106bp Ta: 54ºC *ant[4′]-Ia* 134bp; Ta: 58ºC	Aminoglycosides *aac[3]-Ia* [36] *aac[6′]-Ib* [36] *aph[2″]If* [37] *aac[6′]-Ie/aph[2″]Ia* [37] *ant[4′]-Ia* [38]

a Used in Gram (+) resistant microorganisms. b Resistant *S. aureus*. c Gram (-) resistant microorganisms.

Resistance profiles associated with HAI

The antibiotic groups of interest evaluated in gram-negative bacteria (Table 4), for *Pseudomonas aeruginosa* (the most prevalent) revealed elevated resistance values of 20-30% against carbapenems; ≥10% against aztreonam, 3rd and 4th-generation cephalosporins, and β-lactamase inhibitors. *P. aeruginosa* exhibited its greatest resistance in VAP, presenting some degree of resistance against all the antibiotics tested, with values of 30-45% against carbapenems and colistin. *Klebsiella pneumoniae* presented resistance of 40% against ampicillin/sulbactam; in VAP this resistance reached 50%, followed by 46.2% against meropenem and up to 37% against other ß-lactams. In CLABSI and CAUTI, *K. pneumoniae* maintained resistance percentages of 15-50% against the majority of ß-lactams tested. *Escherichia coli* exhibited its greatest resistance against ampicillin/sulbactam, with 41% general resistance and >40% resistance in VAP and CLABSI. The resistance against quinolones in *E. coli* were higher in CLABSI (47.6%). In *Staphylococcus aureus* its resistance against oxacillin was close to 30% in VAP and CLABSI. (Table 5).

Genotyping of microorganisms isolated in patients with HAI in Cartagena, Colombia

In gram-negative strains, resistance genes associated with extended spectrum β-lactamases (ESBL) were identified in the most prevalent strains (*P. aeruginosa, K. pneumoniae, E. coli*). In *P. aeruginosa*, genes associated with ESBL such as *TEM, SHV, CTX-M* were identified in values that ranged between 15-32% as well as KPC carbapenemases, which were found at a 5.7% frequency (Tables 6 and 7).

Oxacillinase type *OXA-48* was recognized exclusively in *P. aeruginosa* (1.8%). In contrast, *OXA-23* was identified in both *P. aeruginosa* (8.8%) and *E. coli* (2.6%). *OXA-1, OXA-4 and OXA-30* were found with values of 20-50% in the gram-negatives evaluated.

PMQR resistance (quinolones) related to *qnrB, qnrS* and *qnrD* genes was detected in greater proportions in resistant *E. coli* strains. The *aac(6')*-Ib-cr gene of the PMQR type was also found in *E. coli* at a 23.1% frequency. Genes coding for *oqxA* and *oqxB* efflux pumps were identified in *P. aeruginosa, K pneumoniae* and *E. coli* (Table 6).

Table 2. Sociodemographic characteristics of the population monitored for HAI in Cartagena, Colombia.

Characteristic	Patients	VAP[a]	CLABSI[b]	CAUTI[c]	SSI[d]
Sample distribution: N (%) Number of monitored patients Number of monitored events	 199 218	 76 (38.3%) 83 (38.1%)	 65 (32.7%) 70 (32.1%)	 37 (18.6%) 43 (19.7%)	 21(10.6%) 22 (10.1%)
SEX: N (%) Male Female	 **100 (50.3%)** 99 (49.7%)	 44 (57.9%) 32 (42.1%)	 29 (44.6%) 36 (55.4%)	 20 (54.1) 17 (45.9)	 7 (33.3%) 14 (66.7%)
AGE: (mean in years and SD)	**59.6** (19.5)	58.9 (20.7)	59.7 (18.7)	58.9 (21.1)	**62.6** (14.4)
Patient in isolation: N (%) YES NO NS	 8 (4.2%) **189 (95%)** 2(0.8)	 6 (7.2%) 70 (92.1%)	 0 65 (100%)	 2 (5.4%) 34 (91.9%) 1 (4.8%)	 0 20 (93.2%) 1 (4.8%)
Type of ICU N (%) Intensive Intermediate	 **162 (81.4%)** 37 (18.6%)	 70(92.1%) 6 (7.9%)	 47 (72.3%) 18(27.7%)	 29 (78.4%) 8 (21.6%)	 16 (76.2%) 5 (23.8%)
Time spent in ICU until diagnosis of HAI (days) - median [IQR]	4 (2-9)	4 (2-7)	5 (2-13)	4 (2-8)	3 (0-12)
Outcome: N (%) Death Recovery NS	 25 (12.6%) **156 (78.3%)** 18 (9%)	 13 (17.1%) **56 (73.7%)** 7 (9.2%)	 6 (9.2%) 52 (80%) 7 (10.8%)	 5 (13.5%) 30 (81.1%) 2 (5.4%)	 1 (4.8%) 18 (85.7%) 2 (9.5%)
HAI as cause of death: N (%) YES NO	 **23 (92%)** 2 (8%)	 12 (92.3%) 1(7.7%)	 5 (83.3%) (16.7%)	 5(100%) 0	 1(100%) 0
Prevalence % (95% CI)	41.4 (36.9-45.9)	15.8 (12.7-19.4)	13.5 (10.6-16.9)	7.7 (5.5-10.5)	4.4 (2.7-6.6)
Number of Events per hospitalization: N (%) 1 event > 1 event	 180 (90.5%) 19 (9.5%)	 69 (90.8%) 7 (9.2%)	 60 (92.3%) 5 (7.7%)	 31 (83.8%) 6 (12.2)	 20 (95.2%) 1 (4.8%)
Diagnosis at admission: N (%) Infectious Medical Diagnosis Non-Infectious Medical Diagnosis Major Surgery No data	 84 (42.2%) 80 (40.2%) 33 (16.6%) 2 (1%)	 33 (43.4%) 35 (46.1%) 7 (9.2%) 1 (1.3%)	 31 (47.7%) 25 (38.5%) 9 (13.8%) -	 13 (35.1%) 17 (45.9%) 6 (16.2%) 1 (2.7%)	 7 (33.3%) 3 (14.3%) 52.4%) -

[a] Ventilator-Associated Pneumonia. [b] Central Line-Associated Bloodstream Infection. [c] Catheter-Associated Urinary Tract Infection. [d] Surgical Site Infection.

Table 3. Phenotypic characterization of the pathogens identified in patients with HAI.

Microorganism*	n	% (95% CI)	VAP[b] % (95% CI)	CLABSI[c] %(95% CI)	CAUTI[d] %(95% CI)	SSI[e] %(95% CI)
Gram Negative						
P. aeruginosa	**57**	**19.1 (14.8 - 23.9)**	**24.3 (16.5 – 33.5)**	**18 (11.4 – 26.4)**	**12.7 (5.3 – 24.5)**	**15.4 (4.4 – 34.9)**
K. pneumoniae	**40**	**13.4 (9.7 - 17.8)**	**13.1 (7.3 – 21)**	**16.2 (1 – 24.4)**	**10.9 (4.1 - 22.2)**	**7.7 (1 – 25)**
E. coli	**39**	**13 (9.4 - 17.4)**	**6.5 (2.7 – 13)**	**18.9 (12.1 – 27.4)**	**18.2 (9.1- 31)**	3.8 (1 – 19.6)
P. mirabilis	11	3.7 (1.8 - 6.5)	1.9 (0.2 – 6.6)	2.7 (0.6 – 7.7)	9.1 (3 - 20)	3.8 (1 – 19.6)
A. baumanii	9	3 (1.4 - 5.6)	1.9 (0.2 – 6.6)	4.5 (1.5 – 10.2)	1.8 (0.1 – 9.7)	3.8 (1 – 19.6)
E. cloacae	7	2.3 (0.9 - 4.7)	1.9 (0.2 – 6.6)	2.7 (0.6 – 7.7)	1.8 (0.1 – 9.7)	3.8 (1 – 19.6)
S. maltophilia	6	2 (0.7 - 4.3)	0.9 (0.02 – 4.9)	2.7 (0.6 – 7.7)	1.8 (0.1 – 9.7)	3.8 (1 – 19.6)
A. xylosoxidans	4	1.3 (0.4 – 3.4)	3.7 (1 – 9.3)	-	**-**	**-**
K. oxytoca	4	1.3 (0.4 – 3.4)	**-**	2.7 (0.6 – 7.7)	1.8 (0.1 – 9.7)	**-**
Other [a]	31	10.4 (7.2 – 14.4)	13.1 (7.3 – 21)	6.3 (2.6 – 12.6)	**10.9 (4.1 - 22.2)**	15.4 (4.4 – 34.9)
Gram Positive						
S. aureus	**13**	**4.3 (2.3 – 7.3)**	**6.5 (2.7 -13)**	**5.4 (2 – 11.4)**	-	-
S. epidermidis	**5**	**1.7 (0.5 - 3.8)**	-	**3.6 (1 -9)**	1.8 (0.1 – 9.7)	-
S. hominis	**5**	**1.7 (0.5 - 3.8)**	**1.9 (0.2 – 6.6)**	2.7 (0.6 – 7.7)	-	-
E. faecalis	4	1.3 (0.4 – 3.4)	0.9 (0.02 – 4.9)	-	**5.5 (1.1 – 15.1)**	-
E. casseliflavus	3	0.9 (0.02 – 4.9)	0.9 (0.02 – 4.9)	0.9 (0.02 – 4.9)	**-**	**3.8 (1 – 19.6)**
Other [a]	8	2.7 (1.2 – 5.2)	1.9 (0.2 – 6.6)	1.9 (0.2 – 6.6)	1.8 (0.1 – 9.7)	7.7 (1 – 25)
Yeasts						
C. famata	**6**	**2 (0.7 – 4.3)**	**3.7 (1 – 9.3)**	0.9 (0.02 – 4.9)	-	**3.8 (1 – 19.6)**
C. albicans Other [a]	4	1.3 (0.4 – 3.4)	-	0.9 (0.02 – 4.9)	**5.5 (1.1 – 15.1)**	-
	6	2 (0.7 – 4.3)	2.8 (0.6 – 8)	1.9 (0.2 – 6.6)	1.8 (0.1 – 9.7)	-
Negative	28	9.4 (6.3 – 13.3)	9.3 (4.6 – 16.5)	7.2 (3.2 – 14)	9.1 (3 – 20)	19.2 (6.5 – 39.3)

* No sample was available in 3% (9) of the cases. - No microorganisms were isolated. [a] Number of isolates equal to 1 in the respective classification. [b] Ventilator-Associated Pneumonia. [c] Central Line-Associated Bloodstream Infection. [d] Catheter-Associated Urinary Tract Infection. [e] Surgical Site Infection.

In *Staphylococcus aureus,* macrolide resistance related to genes encoding *erm* was founded in 16.7% in CLABSI. Regarding aminoglycosides, *aph[2']'If* and *ant [4']*-Ia were identified in 7.7%. In CLABSI resistance to aminoglycosides was associated with the presence of *aph[2']'If* (25%), *aac[6']-Ie/ aph[2']'Ia* (25%) and *ant[4']-Ia* (25%) (Table 7).

Discussion

This work allowing for the first time in Cartagena a phenotypic and genotypic characterization of the HAIs in all the adult ICUs found that the most prevalent HAIs were VAP and CLABSI. Other national and international studies also describe VAP and CLABSI as the most frequent[11,12]. Since permanent notification of HAI began in Colombia (2013) CLABSI and VAP are the most frequent[13–16]. International series highlight as the most affected population those approximately 60 years of age, with infection times of +/- 6 days[13,17].

This study establishes that 71.7% of the isolates in HAI were gram-negative bacteria (*P. aeruginosa* in VAP, *K. pneumoniae* and *E. coli* in CLABSI and CAUTI) and 13.1% gram-positive bacteria (*S. aureus* in VAP and CLABSI). This behavior is not far from what is reported worldwide. Data from the SIVIGILA surveillance have showed the predominance of these pathogens in national isolates, and in the current updates. In the USA, *E. coli* and *S. aureus* are the most frequent, although *P. aeruginosa and K. pneumoniae* have also been described. In Europe, *P. aeruginosa* leads the headlines in VAP, followed by coagulase-negative *Staphylococcus* in CLABSI and *E. coli* in CAUTI in the same way as the present study. Literature indicates that these pathogens are the most reported and factors like population > 60 years, extended hospital stays, insertion and duration of invasive devices, antibiotic pressure and the breakdown of aseptic measures are associated with HAIs by these microorganisms[5,13–15,17,18].

In the resistance patterns VAP exhibited the highest percentages of resistance, especially in *P. aeruginosa* against to carbapenems, quinolones and colistin. This result is associated with worse outcomes in this type of patient, data are consistent with those reported by surveillance systems such as SENTRY, ECDC and RELAVRA, generating a public health alarm affecting the outcome of these patients[12,19,20].

The genotypic profile showed a wide presence of ESBL associated genes of Ambler class A (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{KPC}) and D (bla_{OXA-1}, bla_{OXA-4}, bla_{OXA-30}, bla_{OXA-48}, bla_{OXA-23}), which have been identified in nosocomial enterobacteria, as in our case, in South America since 1987, in Chile, Buenos Aires [21] and since 2002 in Colombia [22], where they are all widely distributed in the national territory. A profile that drew attention was the simultaneous presence of bla_{TEM}; bla_{SHV}; bla_{CTX-M}; bla_{OXA-1}; bla_{OXA-4} and bla_{OXA-30} in strains of *K. pneumoniae* and *P. aeruginosa* in VAP and CLABSI, confirming the epidemiological behavior of these resistance markers and the poor therapeutic response compromising the outcome of the patients[21,22].
In quinolones draws attention the highest proportion of *qnrS* (12.9%), *qnrD* (15.4%), *aac[6']-Ib-cr* (23.1%) genes in *E. coli*,

Table 4. Resistance profiles of the main gram-negative microorganisms associated with HAI in Catagena, Colombia.

Organism/ Antimicrobial	%R	VAP[a] %R	CLABSI[b] %R	CAUTI[c] %R	SSI[d] %R
P. aeruginosa (n=57)					
Aztreonam	12.3	**23.8**	5	0	-
Ceftazidime	12.3	15.4	15	0	0
Cefepime	14	19.2	10	14.3	-
Imipenem	**21.1**	**34.6**	5	**28.6**	-
Meropenem	**30**	**46.2**	15	**28.6**	0
Piperacillin/Tazobactam	12.3	19.2	10	0	0
Ampicillin/Sulbactam	9	3.8	10	14.3	**25**
Amikacin	10.5	19.2	0	14.3	0
Gentamicin	14	19.2	10	14.3	0
Ciprofloxacin	12.3	**26.9**	0	0	-
Levofloxacin	12.3	**26.9**	0	0	-
Colistin	**31.6**	**46.2**	**20**	**28.6**	-
K. pneumoniae (n=40)					
Aztreonam	15	14.3	16.7	16.7	-
Cefuroxime	**27.5**	**37.1**	16.7	**50**	-
Ceftazidime	15	7.1	**22.2**	16.7	-
Cefepime	18	14.3	**22.2**	16.7	-
Imipenem	5	7.1	5.6	0	-
Meropenem	7.5	**46.2**	15	**28.6**	0
Ertapenem	5	14.3	**33.3**	**33.3**	-
Piperacillin/Tazobactam	-	-	-	-	-
Ampicillin/Sulbactam	**40**	**50**	**33.3**	50	0
Amikacin	2.5	0	5.6	0	-
Gentamicin	**20**	14.3	**22.2**	**33.3**	-
Ciprofloxacin	17.5	7.1	**22.2**	**33.3**	0
Levofloxacin	7.5	0	11.1	16.7	-
Colistin	10	7.1	11.2	16.7	-
E. coli (n=39)					
Aztreonam	5.1	14.3	0	**100**	-
Cefuroxime	**23.1**	**28.6**	**23.8**	10	**100**
Ceftazidime	10.3	14.3	9.5	10	0
Cefepime	7.7	14.3	4.8	0	-
Imipenem	5.1	14.3	4.8	0	-
Meropenem	7.7	14.3	9.5	0	0
Ertapenem	2.6	14.3	**33.3**	**20**	**100**
Piperacillin/Tazobactam	2.6	0	0	0	**100**
Ampicillin/Sulbactam	**41**	**42.9**	**47.6**	**20**	**100**
Amikacin	5.1	14.3	4.8	0	0
Gentamicin	**12.8**	0	**23.8**	0	0
Ciprofloxacin	**17.5**	**28.6**	**42.9**	**30**	0
Levofloxacin	7.5	14.3	0	0	0
Colistin	0	0	0	0	0

0 All strains sensitive to the antibiotic. - Antibiotic not tested. [a] Ventilator-Associated Pneumonia. [b] Central Line-Associated Bloodstream Infection. [c] Catheter-Associated Urinary Tract Infection. [d] Surgical Site Infection.

Table 5. Resistance profiles of the main gram-positive microorganisms associated with HAI.

Organism/ Antimicrobial	%R	VAP[a] %R	CLABSI[b] %R	CAUTI[c] %R	SSI[d] %R
S. aureus (n=13)					
Ampicillin	-	-	-	NA	NA
Oxacillin	30.7	28.6	33.3	NA	NA
Clindamycin	7.7	-	16.7	NA	NA
Erythromycin	23.1	14.3	33.3	NA	NA
Vancomycin	15.4	0	16.7	NA	NA
Gentamicin	7.7	14.3	0	NA	NA
Linezolid	0	0	-	NA	NA
Ciprofloxacin	0	0	-	NA	NA
S. epidermidis (n = 5)					
Ampicillin	-	NA	-	-	NA
Oxacillin	60	NA	50	100	NA
Clindamycin	40	NA	25	100	NA
Erythromycin	60	NA	50	100	NA
Vancomycin	0	NA	0	0	NA
Gentamicin	20	NA	25	-	NA
Linezolid	0	NA	0	-	NA
Ciprofloxacin	40	NA	35	100	NA
S. hominis (n=5)					
Ampicillin	100	100	100	NA	NA
Oxacillin	60	50	66.7	NA	NA
Clindamycin	80	100	66.7	NA	NA
Erythromycin	80	100	66.7	NA	NA
Vancomycin	20	100	66.7	NA	NA
Gentamicin	60	100	66.7	NA	NA
Linezolid	40	100	0	NA	NA
Ciprofloxacin	60	50	66.7	NA	NA
E. faecalis (n=4)					
Ampicillin	25	100	NA	0	NA
Oxacillin	-	-	NA	-	NA
Clindamycin	-	-	NA	-	NA
Erythromycin	-	-	NA	-	NA
Vancomycin	0	-	NA	0	NA
Gentamicin	25	100	NA	0	NA
Linezolid	50	-	NA	66.7	NA
Ciprofloxacin	-	-	NA	-	NA

0 All strains sensitive to the antibiotic. - Antibiotic not tested. NA This pathogen was not isolated in this type of event. [a] Ventilator-Associated Pneumonia. [b] Central Line-Associated Bloodstream Infection. [c] Catheter-Associated Urinary Tract Infection. [d] Surgical Site Infection.

decreasing the activity of ciprofloxacin and norfloxacin, and strengthened by the presence of *oqxA* (18%) and *oqxB* (7.7%) genes related to the OqxAB efflux pump, widely described against quinolones, posing an unfortunate scenario for this type of drug so useful in these infections[23].

It is concluded that in the monitored population VAP and CLABSI are the most prevalent HAIs whose most frequent etiology is gram-negative bacteria with a resistance profile against most of the antibiotics tested, corroborated by the presence of resistance genes hindering the therapeutic approach and prognosis and generating an alert to the interventions derived from this characterization.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Table 6. Distribution of the resistance genes to ß-lactams, quinolones and aminoglycosides in gram-negative isolates from patients with HAI in Cartagena, Colombia.

Organism/gene	%	VAP[a] %	CLABSI[b] %R	CAUTI[c] %R	SSI[d] %R
P. aeruginosa (n=57)					
TEM	31.6	19.2	45	42.9	25
SHV	28.1	15.4	45	28.6	25
CTX-M	14.4	15.4	10	28.6	25
KPC	5.7	3.9	10	0	0
MTSO	22.8	15.4	35	14.3	25
OXA-48	1.8	3.9	0	0	0
OXA-23	8.8	11.5	10	0	0
qnrB	0	0	-	-	-
qnrS	1.8	33.3	-	-	-
qnrD	1.8	50	0	-	-
aac[6′]-Ib-cr	0	0	-	-	-
oqxA	1.8	50	-	-	-
oqxB	1.8	100	-	-	-
aac[6′]-Ib	0	-	-	0	-
aph[2′′]If	1.8	0	0	14.3	-
aac[6′]-Ie/aph[2′]′Ia	0	-	-	0	-
ant[4′]-Ia	0	-	-	0	-
K. pneumoniae (n=40)					
TEM	57.5	71.4	55.6	33.3	50
SHV	37.5	50	33.3	33.3	0
CTX-M	42.5	50	38.9	33.3	50
KPC	32.5	35.7	27.8	33.3	50
MTSO	50	57.1	55.6	16.7	50
OXA-48	0	0	0	0	0
OXA-23	0	0	0	0	0
qnrB	2.5	0	5.6	-	-
qnrS	0	-	0	-	-
qnrD	0	0	-	-	-
aac[6′]-Ib-cr	2.5	-	5.6	-	-
oqxA	2.5	-	5.6	-	-
oqxB	2.5	-	5.6	-	-
aac[6′]-Ib	7.5	14.3	5.7	-	-
aph[2′′]If	7.5	7.1	11.1	-	-
aac[6′]-Ie/aph[2′]′Ia	5	0	11.1	-	-
ant[4′]-Ia	10	14.3	11.1	-	-
E. coli (n=39)					
TEM	53.9	42.9	57.1	60	-
SHV	**10.3**	14.3	9.5	10	-
CTX-M	**10.3**	14.3	14.3	0	-
KPC	**5.1**	14.3	4.8	0	-
MTSO	**28.2**	14.3	33.3	30	-
OXA-48	**0**	0	0	0	-
OXA-23	**2.6**	0	0	10	-
qnrB	**2.5**	14.3	0	0	-
qnrS	**12.9**	14.3	19.1	-	-
qnrD	**15.4**	14.3	14.3	20	-
aac[6′]-Ib-cr	**23.1**	14.3	28.6	20	-
oqxA	**18**	14.3	19.1	20	-
oqxB	**7.7**	0	14.3	-	-
aac[6′]-Ib	**12.8**	-	23.8	-	-
aph[2′′]If	**7.7**	0	14.3	-	-
aac[6′]-Ie/aph[2′]′Ia	**7.7**	-	14.3	-	-
ant[4′]-Ia	**7.7**	-	14.3	-	-

0 Resistance gene not detected. - Gene not tested. [a] Ventilator-Associated Pneumonia. [b] Central Line-Associated Bloodstream Infection. [c] Catheter-Associated Urinary Tract Infection. [d] Surgical Site Infection.

Table 7. Distribution of resistance genes to erythromycin, clindamycin, β-lactams, aminoglycosides, and quinolones in isolates of S. aureus and S. epidermidis in patients with HAI.

Organism/gene	%	VAP [a] %	CLABSI [b] %R	CAUTI [c] %R	SSI [d] %R
S. aureus (n=13)					
ermA	0	0	0	0	0
ermC	7.7	0	16.7	0	0
mecA	0	0	0	0	0
aac[3]-Ia	-	-	-	-	-
aph[2′′]If	7.7	14.3	-	-	-
aac[6′]-Ie/aph[2′]′Ia	0	0	-	-	-
ant[4′]-Ia	7.7	14.3	0	-	-
qnrS	0	-	0	-	-
qnrD	-	-	-	-	-
aac[6′]-Ib-cr	-	-	-	-	-
qepA	0	-	0	-	-
oqxA	-	-	-	-	-
oqxB	-	-	-	-	-
S. epidermidis (n = 5)					
ermA	0	0	0	-	-
ermC	40	-	25	100	-
mecA	0	0	0	0	0
aac[3]-Ia	0	-	0	-	-
aph[2′′]If	20	-	25	-	-
aac[6′]-Ie/aph[2′]′Ia	20	-	25	-	-
ant[4′]-Ia	20	-	25	-	-
qnrS	40	-	25	100	-
qnrD	20	-	0	100	-
aac[6′]-Ib-cr	20	-	0	100	-
qepA	40	-	25	100	-
oqxA	40	-	25	100	-
oqxB	40	-	25	100	-

0 Resistance gene not detected. - Gene not tested. [a] Ventilator-Associated Pneumonia. [b] Central Line-Associated Bloodstream Infection. [c] Catheter-Associated Urinary Tract Infection. [d] Surgical Site Infection.

Right to privacy and informed consent. The authors declare that no data that enables identification of the patients appears in this article.

Conflict of interest. The authors declare that the revision was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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