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Negative correlation between virulence and multidrug resistance in intrahospital and community acquired infections by *Proteus mirabilis*, in Eastern Venezuela.

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Key words: *Proteus;* MDR; virulence; resistance; antimicrobials.

Abstract. This is the first report for Venezuela of virulence/pathogenicity and resistance factors in intrahospital (HCAI) and community-acquired infections (CAI) by *P. mirabilis* in two main hospitals from Eastern Venezuela. Virulence factors such as motility, biofilms, and resistance to serum killing (RSK) were determined. Antimicrobial susceptibility allowed classifying the isolates into resistant, multidrug resistant (MDR) and extensively drug-resistant (XDR). *P. mirabilis* was identified in HCAI in both hospitals mostly from secretions, while some CAI were identified from urine and secretions. Twitching, swarming, biofilm and RSK were identified in many isolates. Eleven antimicrobials showed resistance frequencies from 22-54% in one or both hospitals. A high frequency of MDR isolates was found in these hospitals (60.6 to 56.5%). Strains carrying both $bla_{\text{CTX-M}}$ and bla_{TEM} genes were found in one hospital in a frequency of 27.0%. We also found that the frequency of MDR was lower in strains with three or more virulence factors compared to those with fewer factors. Bacteria with swarming showed 5.85 times lower probability of being MDR, and those with twitching, 7.52 times lower probability. Infections by MDR/XDR *P. mirabilis* strains in HCAI and CAI represent a public health problem that requires effective control and prevention measures to reduce their potential spread and persistence in the population.

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Correlación negativa entre la viruluencia y la resistencia multidroga en infecciones intrahospitalarias y adquiridas en la comunidad por *Proteus mirabilis*, en el Oriente de Venezuela.

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Palabras clave: *Proteus;* MDR; virulencia; resistencia; antimicrobianos.

Resumen. Este es el primer reporte para Venezuela de virulencia/patogenicidad y factores de resistencia en infecciones intrahospitalarias (IAAS) y adquiridas en la comunidad (IAC) por *P. mirabilis* en dos hospitales principales del oriente de Venezuela. Se determinaron factores de virulencia como la motilidad, formación de biopelícula y resistencia al suero humano normal (RSHN). La susceptibilidad a los antimicrobianos permitió clasificar los aislamientos en resistentes, multirresistentes (MDR) y extensivamente resistentes a fármacos (XDR). Se identificó *P. mirabilis* en IAAS en ambos hospitales principalmente a partir de secreciones, mientras que algunos IAC se identificaron en orina y secreciones. Se identificaron motilidades "twitching" y "swarming", biopelículas y RSHN en muchos de los aislamientos. Once antimicrobianos mostraron frecuencias de resistencia del 22 al 54% en uno o ambos hospitales. En estos hospitales se encontró una alta frecuencia de aislamientos MDR (60,6 a 56,5%). En un hospital se encontraron cepas que portaban genes $bla_{\text{CTX-M}}$ y bla_{TEM} con una frecuencia del 27,0%. También encontramos que la frecuencia de MDR fue menor en las cepas con tres o más factores de virulencia en comparación con aquellas con menos factores. Las bacterias con "swarming" mostraron una probabilidad 5,85 veces menor de ser MDR, y aquellas con "twitching", una probabilidad 7,52 veces menor. Las infecciones por cepas de *P. mirabilis* MDR/XDR en IAAS y IAC representan un problema de salud pública que requiere medidas de control y prevención efectivas para reducir su potencial propagación y persistencia en la población.

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INTRODUCTION

P. mirabilis is recognized as an etiological agent of different infectious processes, and its colonization capacity is due to the presence of fimbriae that favor the adherence of this bacterium to the renal epithelium, the phenomenon of swarming (SW), hemolytic activity, hydrolysis of urea, deamination of amino acids, proteases, endotoxins and lipopolysaccharides (LPS), among other virulence factors that allow species of the genus *Proteus* to cause more than 40% of intrahospital infections of the urinary tract, most in patients with a urinary catheter (1, 2).

In *Proteus* species, the combined action of adhesins, SW and urease promote entry into the urinary tract, and the bacterium can modulate the expression of specialized virulence factors for its survival. Furthermore, SW facilitates migration through different types of urinary catheters, suggesting that

this movement may play an important role in the initiation of catheter-associated urinary tract infections and along with the biofilm the establishment of the infection. *P. mirabilis* shows the capacity to form biofilms on biological surfaces and abiotic environments (polystyrene, glass, latex, silicone), the latter representing the main cause of 65% of intrahospital infections (3).

Although *P. mirabilis* is naturally resistant to penicillin G, oxacillin, macrolides, lincosamides, streptogramins, glycopeptides, rifampicin, and fusidic acid, this microorganism is still susceptible to many categories of antimicrobials used in clinical practice (4). Data from the SENTRY antimicrobial susceptibility program in the United States and the European Union for the isolates collected in 2009–2011 reported that <10% of the isolates were resistant to amikacin, aztreonam, cefepime, ceftazidime, ceftriaxone, meropenem, and piperacillin/tazobactam (5). However, extended spectrum beta-lactamases (ESBL) constitute a major therapeutic and epidemiological problem, because the presence of these enzymes leads to bacterial multidrug resistance (6), and in *P. mirabilis* the detection of ESBL has increased, reporting in different countries in South America, including: Brazil, Argentina, and Peru (7–9).

The development of multidrug resistance (MDR) and the invasiveness of *Proteus* spp. is related to many virulence factors, such as SW motility, biofilm formation, hemolysins, urease production, and LPS, among others, which can act independently or in complementary fashion, and allow species of this genus a better adaptation to the conditions of the hospital environment, as well as, evading the defense mechanisms of the host for its survival.

This is the first study in Venezuelan hospitals that focuses on evaluating the presence of virulence factors and their relationship with antimicrobial susceptibility in *Proteus mirabilis* clinical isolates. This will allow the assessment of the extent of the virulence and resistance problem that contribute to therapeutic failure, due to the lack of efficacy of antimicrobials; and to be able to establish effective prevention and control strategies in the current poor conditions of the Venezuelan health system.

MATERIALS AND METHODS

Samples

Bacteria of the Enterobacteriaceae and Morganellaceae families were isolated from clinical samples from patients treated in the two central hospitals of Sucre state, Eastern Venezuela: Hospital Universitario "Antonio Patricio de Alcala" (HUAPA) of Cumana (423 isolates) and Hospital Universitario "Santos Anibal Dominicci" (HSAD) of Carupano (274 isolates), from January through December of 2018. The patients gave their written consent after they were informed of the risks and benefits of participating in the study and had answered a clinical-epidemiological questionnaire. The treatment of the patient's data, the analysis of the isolates, and the information generated were conducted according to the bioethical and biosafety guidelines set out by the Commission on Ethics, Bioethics, and Biodiversity of the Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas "Dra. Susan Tai" at the Universidad de Oriente, Venezuela.

Each clinical isolate was conserved in LB medium, reactivated in brain heart infusion broth (BHI), incubating it for 1 hour at 37°C, and then plated on MacConkey agar, blood agar (BA) and CLED agar, incubating it for 18 hours at 37°C in an aerobic environment. Colonies morphologically suggestive of *P. mirabilis* were selected, and confirmed biochemically by means of the conventional identification protocols. The Urea Broth (Sigma-Aldrich) was used to test the ability to hydrolyze urea to ammonia and carbon dioxide by the urease.

Definitions

Infections were classified into a community-acquired (CAI) and healthcare-asso-

ciated (HCAI) following Cardoso *et al.* (10). The different levels of strain resistance were defined following Magiorakos *et al.* (11) as: multidrug resistant (MDR), when a strain has acquired non-susceptibility to at least one agent in three or more antimicrobial categories; extensively drug-resistant (XDR), when it has acquired non-susceptibility to at least one agent in all, but two or fewer antimicrobial categories; and pan drug-resistant (PDR), when it is non-susceptible to all agents in all antimicrobial categories. Resistant bacteria, non MDR, were those strains showing resistance to one or more antimicrobials, but not being classified as MDR, XDR nor PDR.

Production of biofilm

The production of biofilm was assessed according to Kwiecińska-Pirog *et al.* (12). Overnight cultures of the strains (2 μL) were added to TSB supplemented with 1% glucose (198 μL). The biofilm was quantified by measuring the optical density after staining by crystal violet at 570 nm. The *P. aeruginosa* strain (M-PA01) was used as a positive control and sterile broth culture as a negative control to verify sterility and non-specific media components. The cut-off point was calculated using the negative control (NC), considering negative strains those with values < and producers ≥ .

Motility assays

Swarming (SW) was determined with BA prepared with 0.7% agar, inoculating the isolates on the surface of the agar (10 μL). The presence of swimming (SM), was determined with BA containing 0.3% agar and the isolates (10 μL) were inoculated by puncturing the medium, while for twitching (TM), the BA contained 1% agar and isolates were inoculated with a micropipette below the agar layer (10 μL). All the media were incubated for 24 hours at 37°C, the diameters of the SM and SW zones were measured. For the TM measurement, the agar was removed, and the plates were fixed by air-drying, stained with cristal violet and the stained area was measured (13). In all three cases, the *Escherichia coli* ATCC® 25922 strain was used as a negative control. Following the methodology used to determine the biofilm, strains with negative motility were considered when their measurement was < and positive when it was ≥.

Serum bactericidal assay

The resistance to serum killing (RSK) was determined using normal human serum obtained after coagulation whole blood from healthy donors from the HUAPA Blood Bank. A pool of sera was prepared, aliquoted and stored in 200 μL portions at -80°C. Each bacterial inoculum, of approximately $1x10^7$ CFU/mL, was mixed with an equal volume of serum and incubated for 3 hours at 37°C. RSK was determined immediately after mixing the bacteria with the serum (T_0), and after 3 hours of incubation (T_3), performing the plate dilution quantification method. For this, different dilutions of each isolate ($1x10^{-1}$ - 10^{-4}) were prepared, spreading a 10-μL aliquot of the dilution on the surface of a CLED agar plate, which was incubated at 36°C for 24 hours. Subsequently, the number of colonies was quantified taking the number of T_0 colonies as 100%. The isolates were considered RSK when the number of T_3 colonies was ≥50% with respect to T_0 (14).

Antimicrobial susceptibility assay

Antimicrobial susceptibility was performed using the disc diffusion method, following the guidelines proposed by the CLSI (15). The following antimicrobial categories were tested: Penicillins (ampicillin, AMP: 10 μg), Monobactams (aztreonam, ATM: 30 μg), Cephamycins (cefoxitin, FOX: 30 μg), 1st and 2nd generation Cephalosporins (cephalothin, CF: 30 μg; cefuroxime, CXM: 30 μg), 3rd and 4th generation Cephalosporins (cefotaxime, CTX: 30 μg; ceftriaxone, CRO: 30 μg; ceftazidime, CAZ: 30 μg; cefepime, FEP: 30 μg), Carbapenems (imipenem, IMP: 10 μg; meropenem, MEM: 10 μg; ertapenem, ETP:

10 μg), Betalactamase Inhibitors (amoxicillin / clavulanic acid, AMC: 30 μg; ampicillin/sulbactam, SAM: 20 μg; piperacillin/tazobactam, TZP: 10 μg), Aminoglycosides (gentamicin, GM: 10 μg; amikacin, AK: 30 μg; netilmicin, NET: 30 μg; tobramycin, NN: 30 μg), Fluoroquinolones (ciprofloxacin, CIP: 5 μg), Inhibitors of Folic Acid Metabolism (sulfamethoxazole/trimethoprim, SXT: 1.25 μg/23.75 μg) and Fenicols (chloramphenicol, C: 30 μg). Antimicrobial resistance profiles were established according to Magiorakos *et al*. (11). The quality control of biochemical tests and antimicrobial discs were verified with the control strains of *E. coli* ATTC® 25922 and *P. aeruginosa* ATTC® 27853, from the Venezuelan Center for Microbiological Collections.

Phenotypic and molecular detection of ESBLs

P. mirabilis strains were tested for the phenotypic production of extended spectrum betalactamases (ESBLs) using the modified method proposed by Poulou *et al*. (16). For this, discs of cefotaxime (CTX, 30 μg) and ceftazidime (CAZ, 30 μg) were used, with or without clavulanic acid (CA, 10 μg), both discs with boronic acid (BA, 400 μg) and EDTA (292 μg). An increase of ≥5 mm in the diameter of the inhibitory zone in either CTX/CA or CAZ/CA was considered a positive result for ESBL production.

DNA extraction was carried out using pure *P. mirabilis* overnight cultures. A Wizard Genomic DNA purification kit (Promega Biotech, Spain) was used for extraction, according to the manufacturer's specifications. ESBL producing genes were identified by PCR for bla_{TEM}, bla_{SHV} and bla_{CTX-M} using the primers described previously (17-19). As a positive control, for the bla_{SHV}, bla_{TEM}, and bla_{CTX-M} genes, the *K. pneumoniae* strain ATCC 77915 was used. Amplified products were run in 2% agarose gels, and stained with GelGreen (Biotium, UK). The resulting bands were visualized and photographically documented in an iBright CL1000 (Invitrogen, USA).

Statistical analysis

The data were expressed in tables and figures. The relationship between virulence factors and resistance profiles of the isolates were analyzed by binary logistic regression with the SPSS statistical program, version 18.

RESULTS

From the 423 isolates of Enterobacteriaceae y Morganellaceae analyzed from HUAPA (Cumana), 46 strains were identified as *Proteus mirabilis* (11.1%), while out of 274 isolates of Enterobacteriaceae y Morganellaceae from HSAD (Carúpano), 33 strains (12.4%) were found to be *P. mirabilis*.

In both hospitals, *P. mirabilis* were mainly found in long-stay hospital services such as medicine, trauma surgery, ICU, pediatrics, neonatology, etc, and were classified as HCAI (76.1% (35/46) and 72.7% (24/33), in HUAPA and HSAD, respectively). The HCAI isolates were more frequently found in secretions of different types for both hospitals (76.1% (35/46) and 45.5% (15/33), in HUAPA and HSAD, respectively). *P. mirabilis* caused CAI in nine cases with strains isolated from urine (6) and secretion samples (3) in HSAD, while in HUAPA, there were 11 CAI from strains isolated also from urine (6) and secretion samples (5).

No association was found with the type of infection and the clinical factors of the patients. Only three patients had kidney stones reported in HUAPA, while no patients had septic shock reported. Only one patient from the ICU in HSAD had a recurrent infection, with the strain isolated in day 10 of hospital stay being resistant to AMP, AMC, IMP, GM, NN, CIP, and the strain isolated in day 16 being resistant to the same antimicrobial, as well as to CF, STX and MEM:

The distribution of the SW, TW, and SW measurements showed a pattern that allowed clear differentiation of positive and negative strains, according to the established cut-off points, taken from the measurements of the negative controls (Fig. 1). SW motility

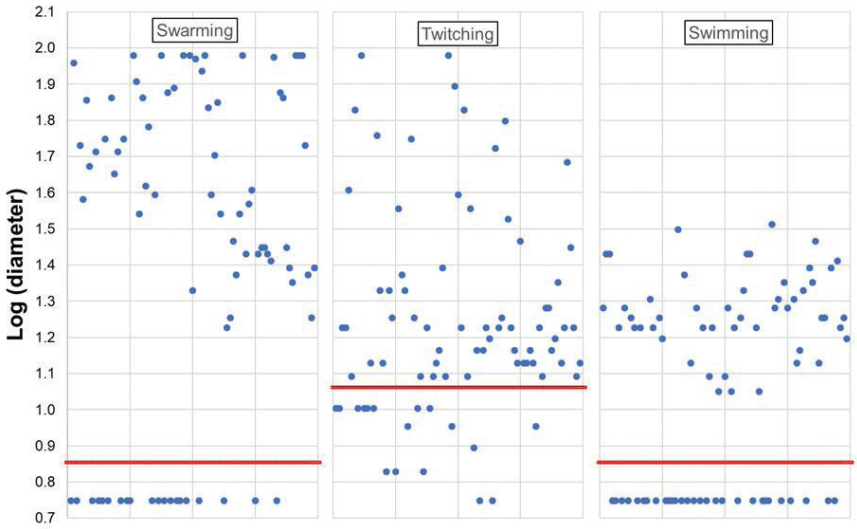

Fig. 1. Distribution of the diameters (mm) shown by the *P. mirabilis* strains in the motility assays. The red lines represent the threshold calculated as described in the text. The diameters were converted into for a better display of the data.

in *Proteus* isolates showed a pattern composed of several concentric rings, where the swarming and consolidation phases are clearly identified (S and C, respectively), but with great morphological variability (Fig. 2). Interestingly, 22/46 isolates of *P. mirabilis* from HUAPA showed all three types of movement, while only 3/33 from HSAD simultaneously showed all three.

About half of the *P. mirabiles* isolates showed RSK phenotypes. The serum bactericidal assay proved to be an easy test to apply and to discriminate among those who showed resistance or sensitivity (Fig. 3). Regarding the distribution of virulence factors, the isolates from HUAPA presented higher frequencies of all the virulence factors evaluated, compared to those from HSAD (Fig. 4). In addition, the most frequent virulence factor in HUAPA isolates was TM, followed by SW and biofilm production. Similarly, HSAD isolates show biofilm production as a more frequent factor, followed by TM, SW. Even though RSK was the least frequent factor in the strains from both hospitals, its frequency of higher than 40% is of clinical importance. The frequencies of SW, SM and TW were statistically higher in HUAPA than in HSAD (Table I).

Isolates of *P. mirabilis* showed high resistance to AMP, CF, IMP, AMC, SAM, GM, NET, NN, CIP, SXT and C (Fig. 5), with higher frequencies in isolates from HUAPA for CF, CTX, CRO, FEP, SAM, SXT, and C, while HSAD isolates showed higher frequencies for GM and NN. Overall, 44 strains of *P. mirabilis* were classified as MDR and two as XDR. No statistically significant difference was found in the frequency of MDR/XDR between both hospitals (Fig. 6A, Table I); although no XDR isolates were detected in HSAD. No PDR isolates were found in either of the two hospitals. However, isolates from HSAD showed resistance to 4-6 categories, while those from HUAPA were mostly resistant to 7-10 antimicrobial categories. (Fig. 6B). Of the strains causing CAI, three and eight showed MDR phenotypes in HSAD and HUAPA, respectively. Additionally, three of the non-MDR resistant srtrains causing CAI were resistant to IMP and/or MEM in HSAD.

When analyzing the presence of MDR strains in relation to the presence of virulence factors evaluated (Fig. 7A), a clear

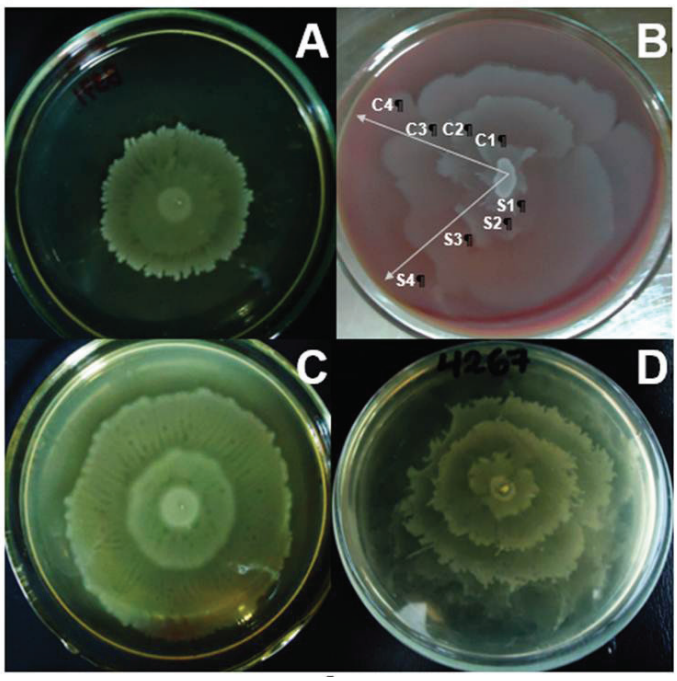

Fig. 2. Different morphology seen in swarming by *P. mirabilis* in Luria Bertani agar (A, C and D), and blood agar (B). Swarming (S) and consolidation (C) phases are shown.

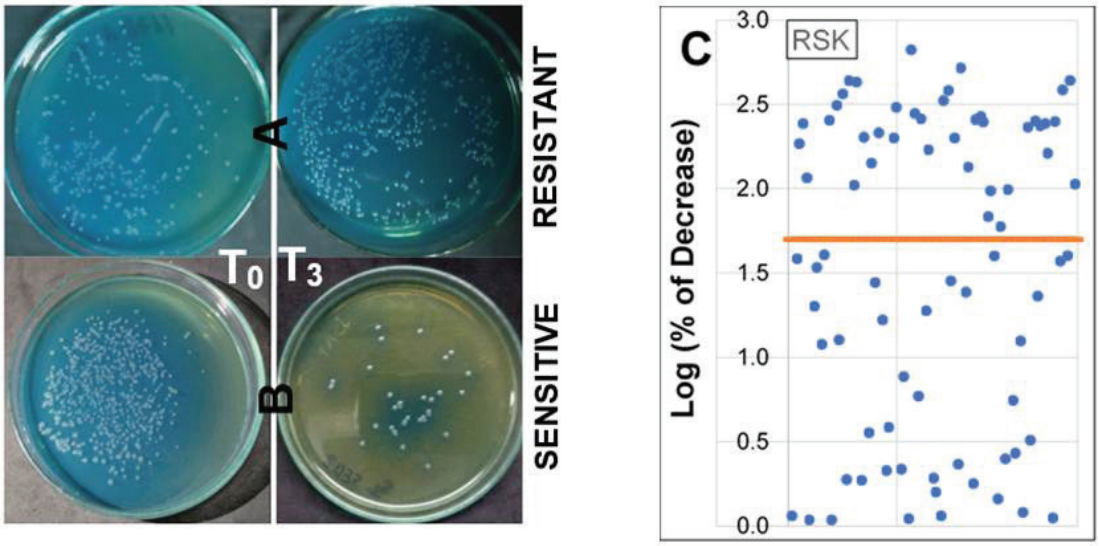

Fig. 3. Resistance to serum killing (RSK) according to the serum bactericidal assay, carried out in cystine–lactose–electrolyte-deficient agar in isolates of *P. mirabilis*. T_0: exposition of the bacteria to human serum without incubation. T_3: exposition of the bacteria after 3 hours of incubation. A: isolate of *P. mirabilis* resistant to serum killing. B: isolate of *P. mirabilis* sensitive to serum killing. C: Distribution of the percentage of reduction (T_3/T_0) shown by the strains, converted into for a better display of the data.

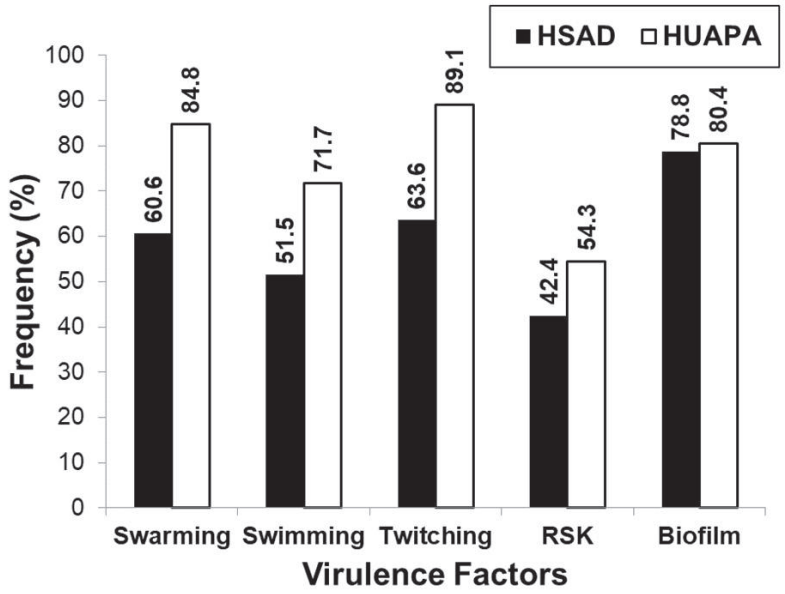

Fig. 4. Frequency of the virulence factors in *P. mirabilis* isolates from patients attending HUAPA and HSAD, Sucre state, Venezuela. HSAD: Hospital "Santos Anibal Dominicci", Carúpano. HUAPA: Hospital Universitario "Antonio Patricio de Alcalá", Cumaná. RSK: Resistance to serum killing according to the serum bactericidal assay.

TABLE I
STATISTICAL ANALYSIS BY BINARY LOGISTIC REGRESSION TO COMPARE THE FREQUENCIES OF VIRULENCE FACTORS IN THE TWO HOSPITALS WHERE THE CLINICAL ISOLATES OF *P. mirabilis* WERE FOUND.

Factor	HSAD	HUAPA	Total	P
SW	20 (60.6%)	39 (84.8%)	59 (74.7%)	0.005*
SM	17 (51.5%)	33 (71.7%)	45 (63.3%)	0.001*
TM	21 (63.6%)	41 (89.1%)	62 (78.5%)	0.013*
Biofilm	26 (78.8%)	37 (80.4%)	63 (79.7%)	0.476
RSK	14 (42.4%)	25 (54.3%)	39 (49.4%)	0.481
MDR/XDR	20 (60.6%)	26 (56.5%)	46 (58.2%)	0.435

P: Probability by binary logistic regression, * P ≤ 0.01, MDR: multidrug and XDR: extensively drug resistant. SW: swarming. SM: swimming. TM: twitching. RSK: Resistance to serum killing according to the serum bactericidal assay.

trend of higher frequency of virulence factors was observed in non-MDR isolates, except for SM, although only statistical significance was observed in SW and TW (Table II). The strains that presented SW or TM were 5.85 and 7.52 times less likely to have MDR/XDR, respectively. Furthermore, the presence of more than two virulence factors in the same strain were significantly negatively associated to the presence of MDR (Fig. 7B); finding that such strains were 5.59 times less likely to have MDR (Table II). One strain showed ESBL phynotype in HSAD, with amplification of $bla_{\text{CTX-M}}$ gene, while one strain without ESBL

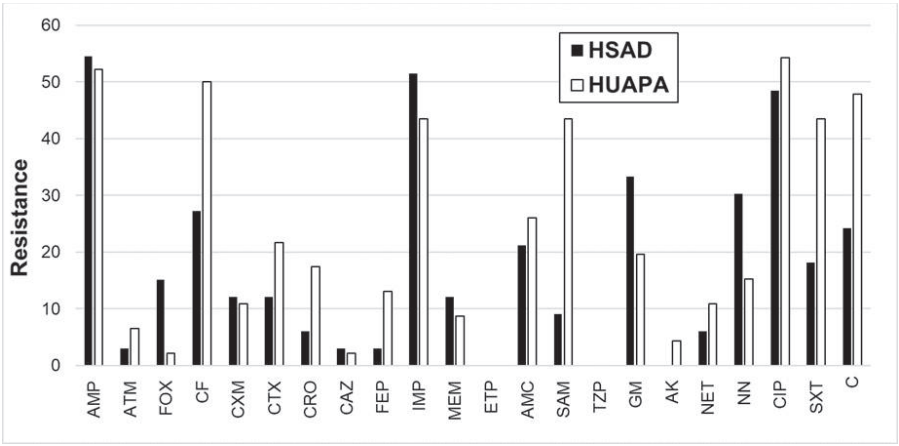

Fig. 5. Frequency of antimicrobial resistance among the strains of *P. mirabilis* isolated from HUAPA and HSAD, Sucre state, Venezuela. Antimicrobial acronysms as described in the materials and methods section.

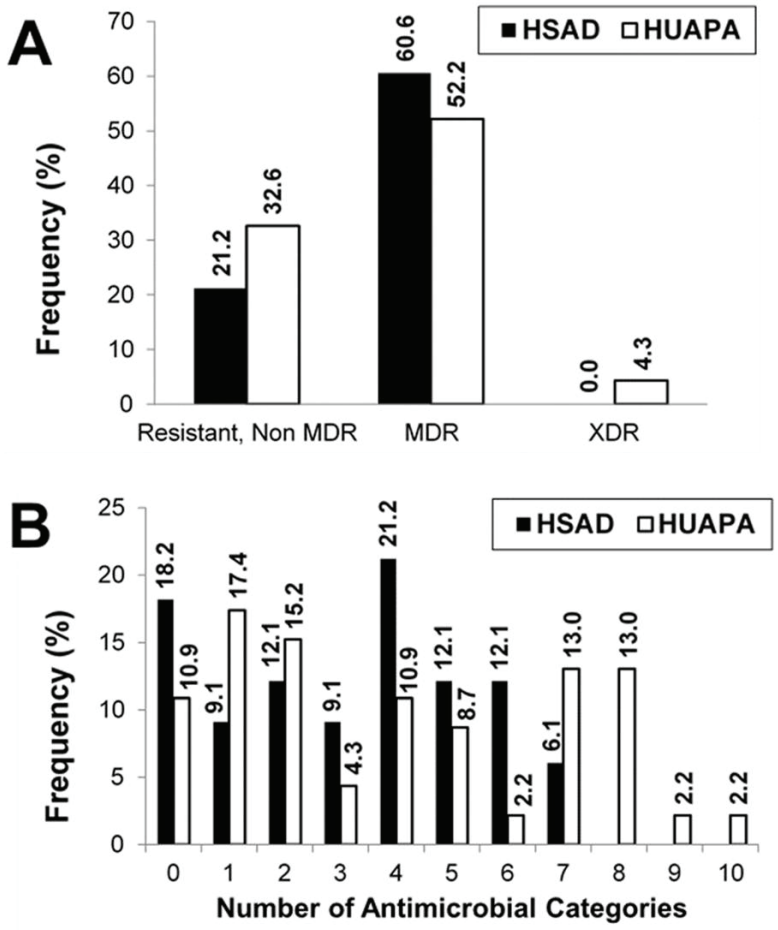

Fig. 6. Antimicrobial resistance (A) and frequency of resistance to different antimicrobial categories (B) of *P. mirabilis* isolates in patients from HUAPA and HSAD, Sucre state, Venezuela. Resistant, Non-MDR: bacteria non-sensitive to two or less antimicrobial categories; MDR: multidrug resistant, non-sensitive to more than two categories; XDR: extensively drug resistant, non-sensitive to all but two or fewer categories, as described in the text.

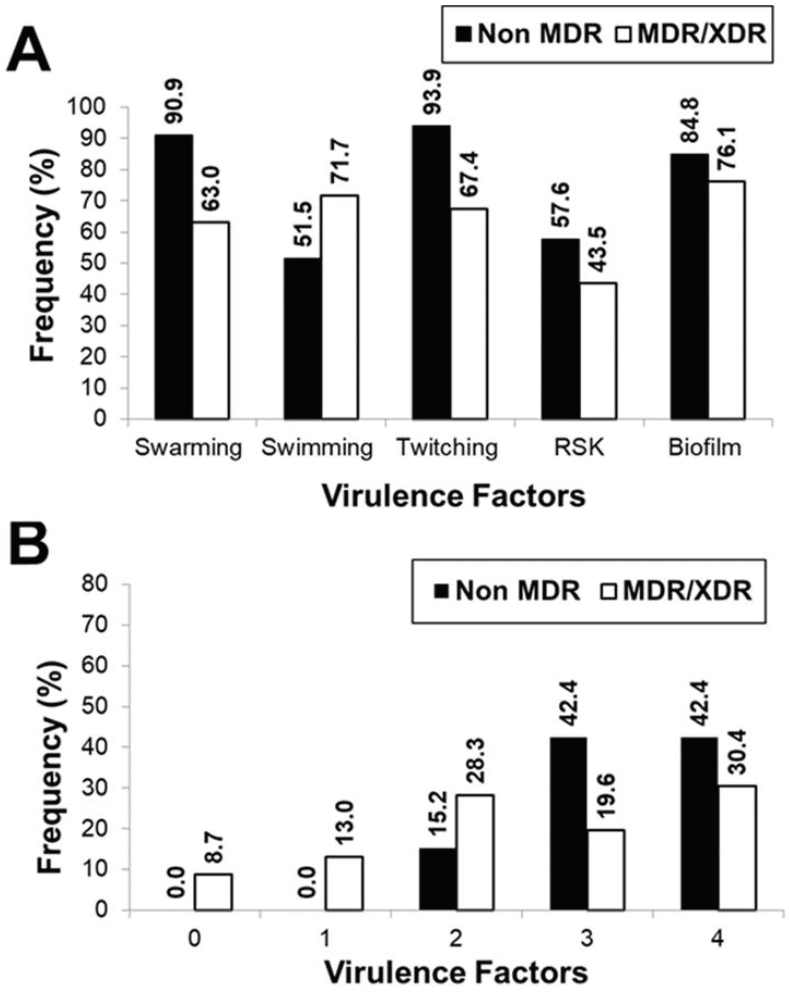

Fig. 7. Relation of multidrug resistance to each of the virulence factors analyzed (A), and the number of virulence factors presented by the isolates of *P. mirabilis* from HUAPA and HSAD, Sucre state, Venezuela. MDR: multidrug and XDR: extensively drug resistant. RSK: Resistance to serum killing, according to the serum bactericidal assay described in the text.

phenotype amplified bla_{TEM} gene in that hospital. Additionally, five strains showed ESBL phynotype in HUAPA, amplifying for both $bla_{C\text{-}TX\text{-}M}$ and bla_{TEM} genes, while four strains with no-ESBL phenotype amplified both of those genes and one all three genes studied.

DISCUSSION

In Latin America, there are medical, social and ecological circumstances that favor a dynamic epidemiology of HCAI and CAI produced by Gram-negative bacteria, which are a public health problem, and their distribution varies among service units in a hospital, with the patient casuistry, sites of infection, antimicrobial administration protocols, types of infections, control practices and local resistance (20–22).

In the central hospitals of the Sucre state, Venezuela: HUAPA and HSAD, despite being geographically separated, showed similar behaviors for *P. mirabilis* infections. In this study, secretions were the main source of isolation of this species in patients with HCAI, while CAI-associated strains were isolated from urine and secretion samples. HCAIs were favored by long hospital stays,

TABLE II
STATISTICAL ANALYSIS BY BINARY LOGISTIC REGRESSION TO COMPARE MULTIDRUG RESISTANCE AND THE VIRULENCE FACTORS IN THE CLINICAL ISOLATES OF *P. mirabilis*.

Factor	Non MDR	MDR/XDR	OR	P
SW	30 (90.9%)	29 (63.0%)	-5,85	0.009*
SM	17 (51.5%)	33 (71.7%)	–	0.068
TM	31 (93.9%)	31 (67.4%)	-7,52	0.010*
Biofilm	28 (84.8%)	35 (76.1%)	–	0.343
RSK	19 (57.6%)	20 (43.5%)	–	0.218
More than 2 Factors	28 (84.8%)	23 (50.0%)	-5,59	0.002*

P: Probability by binary logistic regression, * P ≤ 0.01, OR = odds ratio. MDR: multidrug and XDR: extensively drug resistant. SW: swarming. SM: swimming. TM: twitching. RSK: Resistance to serum killing according to the serum bactericidal assay.

invasive care procedures, among many other factors, and constitute a health problem due to the high mortality they can cause. The higher frequency of *P. mirabilis* in secretions could be due to failures in the availability, use of antimicrobials, the lack of surveillance and epidemiological control of intrahospital infections in these two evaluated health centers, due to poor clinical-epidemiological conditions of the Venezuelan health system. However, similar results have been reported in Ghana (23), who also report *Proteus* in 61.5% of hospital infections and in Bosnia and Herzegovina (24) where this genus was reported as an important intrahospital pathogen in intensive care units, isolated from secretions and urine samples.

Virulence factors in *P. mirabilis* from HUAPA and HSAD show a variable frequency, but the three most frequent factors were TM, SW, and biofilms in both hospitals, and with highly significant differences between HUAPA and HSAD. SW was observed with varied intensity and morphology, and although it is a distinctive behavior of *Proteus*, the relevance of SW patterns is not clear and seems to be more as indicator of environmental factors than a regulatory phenomenon (25, 26). However, the frequency of isolates of *P. mirabilis* not producing SW in strains from both hospitals agree with the studies in the Czech Republic and Poland (13, 14), where 10 and 17%, respectively, of *P. mirabilis* and *P. vulgaris* were found not producing SW. In animal models, some strains of *P. mirabilis* require neither flagella nor SW to cause infection, and swarmer cells are rarely observed during infection, suggesting that their role in virulence and colonization may be tissue-specific (27–29).

The frequency of TM, as well as the simultaneous presence of the three types of motility in 25 isolates of HUAPA and HSAD have also been reported in the Czech Republic (13); but in catheter-associated urinary tract infections, suggesting that these differences may be caused by the growth rates of each isolate, and its ability to adhere to a specific type of catheter or tissue.

Although in *P. mirabilis* isolates from HUAPA and HSAD, no association was shown between the production of SW, SM, and TM with biofilms, the latter being an important virulence factor. The expression of these types of motility, together with the formation of biofilms, can act independently or be expressed simultaneously, allowing *Proteus* strains to a better adaptation to the conditions of the hospital environment, promoting infection in the host, acting as a defense

mechanism for survival and enhancing their virulence. The flagella in SW and TM are known to be involved in surface adhesion and bacterial colonization, which play a critical role in the early stages leading to biofilm formation (30, 31).

Biofilm almost always leads to a significant decrease in antimicrobial sensitivity compared to cultures grown in suspension (32). This, added to the different resistance mechanisms that bacteria exhibit in them, could explain the high frequency of MDR strains in the two hospitals, where resistance affected many antimicrobial categories.

The O-antigen part of the LPS imparts antigenicity to the bacterial cell leading to the production of antibodies. Nonetheless, it should also be noted that RSK, although it was the least frequent virulence factor in *P. mirabilis* for both hospitals, occurred with an important frequency; demonstrating that these isolates are highly pathogenic, because of the variability in the length of the O-antigen chain of the LPS present in these bacteria, can help them avoid the lytic activity of the complement (33). However, in a similar study on *Proteus*, no correlation between the chemical structure of the specific LPS chains and the resistance, or presence of SW was found (14). However, LPS cause *P. mirabilis* to have intrinsic resistance to polymyxins (34), which favors that these MDR strains can significantly increase mortality in patients with severe infections.

The presence of these virulent strains and MDR that are causing HCAI and CAI in patients treated in these hospitals are cause for concern. Mainly those that involve risks of community spread, which could be related to the indiscriminate use of antimicrobials that cause selective pressure on bacteria, leading to a higher prevalence of resistance, which is very common in developing countries like Venezuela.

The Center for Disease Control of USA (CDC) has reported the high increase in antibacterial resistance as one of the most important reasons threatening human health worldwide, and HCAIs are two to three times higher in developing countries, in comparison with Europe or the USA (11, 22). The results found in the *P. mirabilis* isolates show the significant increase in antimicrobial resistance of this genus in the hospitals evaluated in eastern Venezuela. Although the frequency of strains showing ESBL phenotypes in these hospitals was low, the presence of two or more genes that confer resistance to beta-lactams in HUAPA strains, in addition to the high frequency of resistant strains, which was caused by many molecular mechanisms, is a very important finding, with serious repercutions for the health of the population in this region. In fact, according to the SENTRY reports (35), increase in ESBL frequencies are reported for USA (10.9%), Europe (16.2%) and Latin America (22.4%).

When correlating virulence with MDR in *Proteus* isolates from the studied hospitals, virulent and resistant isolates were identified, however, in general, MDR strains presented fewer virulence factors. These results could be explained by the fact that, in some bacterial species, the acquisition of a phenotypic advantage such as antimicrobial resistance (beta-lactamases, porins, efflux pumps, or PBP, among others), is associated with a metabolic or fitness cost, leading to a decrease in virulence and vice versa (36). Although resistance is essential for pathogenic bacteria to overcome antimicrobial therapies, adaptation and survival in challenging hospital environments, it is often associated with a fitness cost motivated by the additional energy expenditure represented by maintenance of these resistance mechanisms or the effects on other essential functions of the cell. Virulence mechanisms, on the other hand, are necessary to overcome host defense systems, favoring the best adaptation of the pathogen, but they also involve a metabolic cost. It can be understood then, that because both adaptation mechanisms require high-energy costs, the strains tend to establish an equilibrium in the number of mechanisms of each type (37). In *Proteus*, it has been reported that the de-

crease or inhibition of SW expression may be associated with the presence of MDR in some strains (38).

This study, carried out on strains of *P. mirabilis* in the hospitals of the state of Sucre, represents the first report for Venezuela of the mechanisms of resistance and virulence, allowing evidence of the prevalence of HCAI by virulent isolates and MDR for this species, which represents a high risk of spread in these hospitals. Furthermore, the presence of strains producing CAI can spread more rapidly in the population, and the isolation of these strains showing MDR phenotypes are great risk for the entire population. It would be very important to be able to deepen the study of possible associations of the factors involved in virulence and antimicrobial resistance, to understand how microorganisms defend themselves during the infection process, allowing the development of effective control and prevention measures to be implemented to prevent the spread of these strains.

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