

URINE INHIBITORY TITERS AGAINST RESISTANT *ESCHERICHIA COLI* ISOLATES AFTER ORAL AMOXICILLIN-SULBACTAM

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Abstract It has been previously shown that following the oral administration of amoxicillin-sulbactam (AXS) the urinary activity against *Escherichia coli* (Ec) is due to beta-lactamase inhibition (i.e. TEM-1) as well as to the intrinsic activity of sulbactam (SB). Similarly, it has been previously demonstrated in volunteers that a single oral dose of AXS 500/500 mg allows high urine inhibitory titers (UITs) against resistant Ec isolates. In this *in vitro* and *ex vivo* study we assessed the urinary activity of a new AXS proportion: 875/125 mg. Urine was collected from 12 volunteers at 0-2; 2-4; 4-6 h after a single oral dose of AXS 875/125mg. Previous studies had shown that pooled urine from 12 volunteers did not differ significantly in the UIT as compared to the mean individual values. Urine pools for each period were prepared. Each pool was tested for UIT against 60 Ec isolates received from 10 different laboratories in South American countries: 10 susceptible (S) to AXS; 10 intermediate (I) and 40 resistant (R); the latter ranging 32/16-256/128 mg/l. Amoxicillin (AX) and SB urine concentrations were determined in all the samples. UIT ranged from 1/4 to >1/32 for S and I strains and from 1/1 to 1/4 for R strains. For one strain (AXS, MIC 256/128 mg/l) the UIT titer was 1/1 at 2 and 4 h but it was not inhibited at 6 h. AX mean levels ranged from 1872 (2 h) to 522 mg/l (6 h) while SB ranged from 1075 (2 h) to 334 (6 h) mg/l. It is noteworthy that 59/60 strains were inhibited by 128 mg/l SB alone. In conclusion: the AXS 875/125 proportion has a remarkable *in vitro* and *ex vivo* activity against Ec urinary isolates.

Key words: amoxicillin-sulbactam (875/125mg), urine inhibitory titers, *Escherichia coli*

Resumen *Título inhibitorio de la orina frente a cepas resistentes de Escherichia coli después de recibir amoxicilina-sulbactam.* Previamente fue demostrado en un grupo de voluntarios que luego de una dosis oral de 500/500 mg de amoxicilina-sulbactam (AXS) la actividad de la orina frente a cepas de *Escherichia coli* (Ec) es debida a la acción inhibitoria de beta-lactamasas (ej: TEM-1) y a la actividad intrínseca de sulbactam (SB), y que dicha administración produce elevados títulos inhibitorios en la orina (TIO), frente a cepas de Ec consideradas resistentes. En este estudio *in vitro* y *ex vivo* verificamos la actividad urinaria de una nueva proporción de AXS: 875/125 mg. Se recolectaron muestras de orina de 12 voluntarios a las 0-2; 2-4 y 4-6 h, luego de una única dosis oral de AXS 875/125 mg. Previamente había sido demostrado que los "pools" de muestras de orina de 12 voluntarios no diferían significativamente en los TIO cuando se compararon con la media de los valores individuales. Por lo tanto se prepararon "pools" de orina para cada período, y cada uno de ellos fue ensayado para determinar los TIO contra 60 cepas de Ec recibidas de 10 laboratorios de países de Sud América: 10 sensibles a AXS; 10 intermedias y 40 resistentes. Estas últimas presentaron un rango de CIM para AXS de 32/16 a 256/128 mg/l. Se determinaron las concentraciones de amoxicilina (AX) y de SB en todas las muestras. Los TIO variaron entre 1/4 y >1/32 para las cepas sensibles o intermedias y de 1/1 y 1/4 para las resistentes. Para una cepa (CIM para AXS 256/128 mg/l) los TIO fueron de 1/1 a las 2 y 4 h pero no hubo acción inhibitoria a las 6h. Los niveles urinarios medios de AXS variaron entre 1872 (2 h) y 522 (6 h) mg/l y para SB entre 1075 (2 h) y 334 (6 h) mg/l. Es de destacar que 59/60 cepas fueron inhibidas por 128 mg/l de SB solo. En conclusión: la proporción AXS 875/125 presenta una relevante actividad *in vitro* y *ex vivo* frente a cepas de Ec aisladas de infecciones urinarias.

Palabras clave: amoxicilina-sulbactam (875/125mg), título inhibitorio de la orina, *Escherichia coli*

It has been shown that beta-lactamase inhibitors, such as sulbactam (SB), exhibit intrinsic activity against highly resistant *Escherichia coli* isolates (i.e. TEM-1 beta-

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lactamase hyperproducers), at the high concentration reached by SB in bladder urine after a single oral dose of 500/500 mg of an amoxicillin-sulbactam (AXS) combination administered to volunteers, as judged by the high urinary inhibitory titers (UITs) obtained in their urine. Furthermore, it was observed that after the administration of SB alone to volunteers, high UITs against resistant *E. coli* could also be obtained¹. Therefore we assumed that the activity of AXS against *E. coli* in bladder urine is not only due to beta-lactamase inhibition but also to the intrinsic activity of SB. This effect is probably due to its binding to high molecular weight *Escherichia coli* Penicillin Binding Protein (PBP)². In a collaborative study performed in 10 centers from 8 South American countries, 200 *E. coli* isolates were collected, which were considered resistant to AXS (and ampicillin-sulbactam or amoxicillin-clavulanate) by both agar diffusion and dilution methods. In that study it also was shown that the isolates were susceptible to the concentrations of amoxicillin (AX) and SB reached in the bladder urine after a single oral dose of amoxicillin 500 mg and sulbactam 500 mg (or its pediatric equivalent of 50 mg/kg/day for each drug)³. In the present *in vitro* and *ex vivo* study we assessed the urinary antimicrobial activity obtained after administration of a new AXS proportion: 875/125 mg against *E. coli* resistant isolates. The aims of this study were: 1. To assess if UITs, obtained in urine after a single 875/125 mg oral dose of AXS administered to volunteers, are high enough to inhibit the growth of *E. coli* resistant isolates. 2. To assess if those UITs are in accordance with the high concentrations of AX and SB achieved in urine and with the AXS and SB MICs of the isolates.

Material and Methods

Ethical issues

The protocol was approved by the Institutional Review Board of the Hospital Manuel Belgrano, San Martín, Buenos Aires. A written informed consent was obtained from all volunteers before being admitted into the study.

Volunteers

Twelve volunteers (six males) aged 23 to 39 years (mean 32) and weighting 54 to 84 kg (mean 68 kg) were included in this study. On admission, volunteers exhibited a disease-free condition; their renal and liver functions were normal and they did not declare any past history of recurrent urinary infection. They had received neither antibiotics for the last 15 days nor other medication in the last 48 hours before entering the study. The day before the study their solid and fluid intakes were standard and adequately documented.

Collection of the baseline urine (hour: 0) previous to the antibiotics administration

A minimum of 30 ml of urine in a sterile, wide opening jar, was obtained aseptically from each volunteer. The sample was

transferred under sterile conditions into a screw-cap sterile tube, and was immediately frozen at -20°C .

Amoxicillin-sulbactam administration

All the volunteers received a single amoxicillin 875mg/sulbactam 125mg-tablet (*Trifamox IBL DUO*, Laboratorios Bagó S.A., Argentina).

Urine samples collection after dosing

Urine samples were collected as follows: sample 1: 2 hours after dosing (0-2 h period); sample 2: 4 hours after dosing (2-4 h period); sample 3: 6 hour after dosing (4-6 h period).

Preparation of a pool of urine samples from each period

In a previous unpublished pilot study we found that UITs determined with pooled urine mixing equal urine volumes from 12 volunteers, for each period, did not significantly differ from the UIT value calculated by averaging the individual UITs. In order to facilitate the assay of a high number of strains, we used pooled urine samples for each period.

UIT determinations

They were performed on every pool, against each selected strain, according to the methodology previously described¹. The baseline urine pool was used to dilute every pool of samples collected at different hourly intervals (samples 1, 2 and 3) from 1/1 to 1/32.

Non-physiological elements such as bacteriologic broth or agar were not used.

For each pool of samples (1, 2 and 3) we prepared the following tube series for which UIT determination were performed:

Tube 0: 0.5 ml of baseline urine pool (growth control); *Tube 1*: 0.5 ml of urine pool after receiving the medication (1/1 dilution); *Tube 2*: 0.5 ml of baseline urine pool to which 0.5-ml of post-antimicrobial urine pool was added (1/2 dilution); *Tube 3*: 0.5 ml of baseline urine pool to which 0.5-ml of tube 2 contents was added (1/4 dilution). And so on up to the 1/32 dilution. Each tube was therefore inoculated with 20 microlitres of a suspension of 10^8 to 10^9 CFU/ml of the *E. coli* strain to be tested. The same procedure was followed on every urine dilution for the 60 strains included in the study and with the *E. coli* ATCC 25922 and *E. coli* ATCC 35218 control strains. The tubes were incubated at 35°C for 16-20 h in air atmosphere. The UIT was considered as the dilution of the prior tube to the first one in which turbidity compatible with bacterial growth was observed, after comparing it with the antibiotic containing urine mixture free of bacteria.

Isolates

Sixty *E. coli* isolates were included in this study: 6 from each of the 10 participant centers (South American Collaborative Study)³. The susceptibility of the received isolates were reassessed in the coordinator center (CEA). They were distributed, according to their susceptibility to AXS by diffusion and dilution methods, into three groups: 10 susceptible strains (MIC < 16/8mg/l); 10 intermediate strains (MIC = 16/8 mg/l) and 39 resistant strains (> 16/8 mg/l). A single isolate from Brazil presented a MIC 256/128 mg/l; which was the highest MIC among these 60 strains.

Determinations of MIC and breakpoints

MICs of AXS (2:1) and SB alone against *E. coli* isolates were determined in accordance to NCCLS recommendation M2-A7⁴

by the agar microdilution using Mueller Hinton agar (*Biokar*. France) and a Steer's replicator devise. Sodium salts of AX and SB were provided by *Laboratorios Bagó S.A.* as pure powders of known potency.

Breakpoints for AXS (mg/l) were the following: susceptible $\leq 8/4$; intermediate 16/8, and resistant $\geq 32/16^5$.

Amoxicillin (AX) and sulbactam (SB) assay

Amoxicillin and sulbactam were assayed by microbiological method: *Sarcina lutea* was used for AX⁶ and a recently developed specific method using *E. coli* ATCC 35218, was used for sulbactam¹.

Results

The UITs of pooled urine samples against *E.coli* with different MIC values are shown in Table 1. The intrinsic activity of SB against 60 *E.coli* isolates is presented in Table 2. Figure 1 shows the mean concentration of AX and SB (mg/l) obtained in the urine samples at different time intervals. UIT ranged at any time after a single oral

TABLE 1.– *Urine inhibitory titers of pooled urine samples against Escherichia coli with different MIC values from volunteers who received amoxicillin-sulbactam (875/ 125 mg) p.o.*

MIC AXS (mg/l)	N° strains	Hour 0	Hour 2*	Hour 4*	Hour 6*
8/4#	10	0	>1/32	>1/16	1/4
16/8	10	0	1/16	1/4	1/4
32/16	23	0	1/4	1/2	1/2
64/32	12	0	1/4	1/2	1/1
128/64	4	0	1/4	1/2	1/1
256/128	1	0	1/1	1/1	NI##

* expressed as mode

Minimal Inhibitory Concentration (MIC): 8 mg/l amoxicillin; 4 mg/l sulbactam

not inhibited

TABLE 2.– *Intrinsic activity of sulbactam (SB) against 60 Escherichia coli isolates included in the study*

MIC (mg/l)	N (isolates)
32	28
64	13
128	18
256	1

Escherichia coli strain ATCC 35218 showed a SB MIC of 32 mg/l

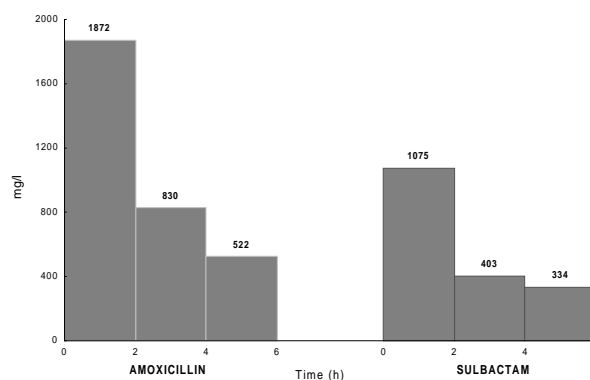


Fig. 1.– Mean concentrations of amoxicillin (AX) and sulbactam (SB) (mg/l) in the volunteers urine samples at different time intervals after a single oral dose of AXS 875/125 mg

dose of AXS 875/125 mg from 1/4 to >1/32 for S and I strains and 1/1 to 1/4 for R strains. For one strain (AXS, MIC 256/128 mg/l) the UIT titer was 1/1 at 2 and 4 h but bacterial growth was not inhibited at 6 h. AX mean levels ranged from 1872 (2 h) to 522 (6 h) mg/l while SB ranged from 1075 (2 h) to 334 (6 h) mg/l. It is noteworthy that 59/60 strains were inhibited by concentrations ≤ 128 mg/l SB alone. (Table 2)

Discussion

The assessment of the inhibitory titers of organic fluids against bacterial isolates with known MICs after antimicrobial drugs administration has proved to be a useful pharmacodynamic parameter to predict their clinical efficacy. In that sense, UITs from volunteer's urine samples against strains of different susceptibility to the drug under study is an appropriate method to verify the bacteriostatic activity of antibacterial drugs, during a certain period of time. This aspect is of great concern for beta-lactam antibiotics because it has been shown that the clinical efficacy of these drugs depends on the maximum length of time during which the antibiotic concentration is sustained over the MIC of infective isolates, even though this approach has only been proposed for respiratory infections^{7, 8}.

For AX and SB, we must consider two reasons in order to predict an inhibitory effect over *E. coli* strains: 1) both are beta-lactam drugs and their activity against *E. coli* in urinary infections does not only rely upon the inhibitory activity of beta-lactamases by sulbactam, but also on the antimicrobial intrinsic activity of SB (without AX) at the urinary level, as we have shown *in vitro* and *ex vivo* in a recently published study¹; 2) both betalactam antibiotics reach a high concentration in the urine.

The long period during which we have observed an effective UIT against all 60 *E. coli* strains is really relevant,

moreover if we consider that 50 of them would have been considered non-susceptible (and 40 of those as resistant) in accordance with the standards of the National Committee for Clinical Laboratory Standards (NCCLS, M100-S12)⁹ applied to ampicillin-sulbactam or amoxicillin-clavulanate serum breakpoints.

Our results differ from the resistance figures that are usually reported in susceptibility tests for *E. coli* strains isolated from urine cultures in Latin America^{10,11} due to the fact that NCCLS breakpoints are determined only on the basis of serum concentrations, without considering either, the urinary levels of amoxicillin and sulbactam or the intrinsic activity of the inhibitor.

The MIC values "at urinary level"^{11,12} for AXS and SB alone are in accordance with the values of the UITs that were found in the urine of those volunteers who received a single dose of amoxicillin-sulbactam (875/125 mg) by the oral route and the urinary concentration of AX and SB matches the UIT values.

These microbiological results are in accordance with the clinical results previously obtained in women with lower urinary tract infections¹³⁻¹⁵. From a therapeutic point of view, it hypothesized that aminopenicillins and cephalosporins, acting on the lactobacillus belonging to the normal vaginal flora, could increase the risk of re-infections; the multiplicity of factors intervening in the colonization of the introitum and urinary tract by *E. coli* would not totally agree with this hypothesis¹⁶. On the other hand, the effectiveness of amoxicillin-sulbactam compared with metronidazol, in women with bacterial vaginosis, has been demonstrated in a recent study¹⁷.

Thus, the *in vitro* and *ex vivo* activity observed in our study provide a pharmacodynamic basis for the therapeutic success of AXS therapy by the oral route, confirming that already published for the treatment of uncomplicated lower urinary tract infections in women and children, on an outpatient basis¹⁸. In conclusion, the AXS 875/125 proportion has a remarkable *in vitro* and *ex vivo* activity against *E. coli* urinary isolates.

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References

- Casellas JM, Visser M, Tomé G, Cohen H, Soutric J, Arenoso H. Erradicación de *Escherichia coli* de la orina vesical por amoxicilina-sulbactam. Actividad intrínseca del inhibidor. *Enferm Infecc Microbiol Clin* 2001; 19: 206-10.
- Livermore DM. Beta-lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557.
- Casellas JM, Visser M, Coco B, et al. South American collaborative study on the etiology of community-acquired lower urinary tract infections and the activity of amoxicillin, amoxicillin-sulbactam and sulbactam. *Revista Chilena de Infectología*. In press 2002.
- National Committee for Clinical Laboratory Standards. 22: M2-A7. 2002. In: NCCLS, Wayne PE, U.S.A.
- Casellas JM, Arenoso HJ, Soutric JL, Tomé G, Goldberg M. Estudio comparativo *in vitro* e *in vivo* de 3 asociaciones de inhibidores suicidas de beta-lactamasas con aminopenicilinas. *Rev Esp Quimioterap* 1993; 6: 289-97.
- Klasen M, Edberg S. Measurement of antibiotics in human body fluids: Techniques and significance. In: Lorian V. *Antibiotics in laboratory medicine*. 4th Ed. Baltimore: Williams & Wilkins. USA. 1996; p 230-295.
- Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis* 1998; 26: 1-10.
- Bantar C, Nicola F, Fernandez Canigia L, et al. A pharmacodynamic model to support a 12-hour dosing interval for amoxicillin/sulbactam, a novel oral combination, in the treatment of community-acquired lower respiratory tract infections. *Journal of Chemotherapy* 2000; 12: 223-7.
- National Committee for Clinical Laboratory Standards. 22: M100-S12; 2002. In: NCCLS, Wayne PE, U.S.A..
- Casellas JM, Guzmán Blanco M, Pinto ME The sleeping giant. Antimicrobial resistance. *Infect Dis Clin North America* 1994; 8: 29-45.
- Guzmán Blanco M, Casellas JM, Sader HS. Bacterial resistance to antimicrobial agents in Latin America: The giant is awakening. *Infect Dis Clin North America* 2000; 14: 67-81.
- Bantar C, Nicola F, Arenoso J, et al. Pharmacokinetic and pharmacodynamics of amoxicillin-sulbactam, a novel aminopenicillin-b-lactamase inhibitor combination, against *Escherichia coli*. *Antimicrob Agents Chemother* 1999; 43: 1503-4.
- Alonso S, Farinati A, Thoroughgood V, Goldberg M, Piccinalli L, Casellas JM. Amoxicilina-sulbactam en infecciones urinarias bajas de la mujer. *Ginecol Reprod* 1990; 2: 27-35.
- Sánchez Velazco H, Biganni L, Delgado E. Tratamiento de la infección urinaria baja aguda de la mujer con monodosis de amoxicilina-sulbactam. *Prensa Med Argent* 1990; 7; 77: 44-7.
- López CR, Contos J. Tratamiento de un día con amoxicilina-sulbactam (infección urinaria baja aguda de la mujer). *Prensa Med Argent* 1992; 79: 17-20.
- Winberg J, Herthelius-Elman M, Mollby R and Nord CE. Pathogenesis of urinary tract infections: experimental studies of vaginal resistance to colonization. *Pediatr Nephrol* 1993; 7: 509-14
- Farinati A, Ortega Soler C, Gambaro E, Mormandi JO, Tilli M, Caruso N. Uso de la combinación amoxicilina-sulbactam en infecciones ginecoobstétricas. Congreso Internacional de Infectología em Ginecología e Obstetricia, Nov 2000, Rio de Janeiro, Brasil. *DST - J Bras Doencas Sex Transm* 2000; 12: 42.
- Exeni R, Casellas JM, Grimoldi I, et al. Efectividad de amoxicilina-sulbactam en el tratamiento de infecciones urinarias. 28° Congreso Argentino de Pediatría, 1988, Buenos Aires, Argentina. En Actas: p 199.