Articolo originale

Original article

Gram-negative urinary tract infections and increasing isolation of ESBL-producing or ceftazidime-resistant strains in children: results from a single-centre survey

Infezioni delle vie urinarie da batteri Gram-negativi e aumento di ceppi ESBL-produttori in pediatria. Risultati di uno studio osservazionale monocentrico

Stefano Giardino[1], Roberto Bandettini[2], Maddalena Perotti[2], Giorgio Piaggio[4], Ludovica Degl'Innocenti[4], Rosanna Sacco[5], Antonella Ciucci[5], Ilaria Caviglia[3], Paola Barabino[6], Francesca Ginocchio[3], Giuseppe Losurdo[3], Riccardo Haupt[7], Elio Castagnola[3]

[1]Stem Cell Transplant Unit, Department of Hematology and Oncology;
[2]Laboratory of Microbiology;
[3]Infectious Diseases Unit, Department of Pediatrics;
[4]Nephrology Unit, Department of Pediatrics;
[5]Hospital Infection Control Team;
[6]Service of Pharmacy;
[7]Epidemiology and Biostatistics Section, Istituto Giannina Gaslini, Genoa, Italy

INTRODUCTION

The emergence of resistant pathogens, especially among Gram-negatives, represents one of the most feared phenomena in recent years. Urinary Tract Infections (UTIs) are frequent in children, and Gram-negative bacteria are the most frequently isolated cause of such diseases.

The high frequency of UTIs in pediatrics is due to the presence of specific risk factors like urinary tract malformation and neurogenic bladder dysfunction, especially in patients affected by spina bifida [1]. Children with these specific risk factors often receive long-term, low-dose antibacterial prophylaxis besides the therapy of specific infectious episodes [1, 2]. The use of wide-spectrum antibiotic prophylaxis and therapies in children with UTIs is probably the most important cause for the development of resistance in Gram-negative rods with the emergence of extended-spectrum beta-lactamase (ESBL) producing or AmpC beta-lactamase cephalosporin-resistant (ceftazidime, CAZ-R) strains [3-5].

In this report we describe the epidemiology of UTIs due to Gram-negative and the occurrence of ESBL and CAZ-R strains observed at the Gaslini Children's Hospital in Genoa during a prospective survey performed from January 2007 to December 2011.

PATIENTS AND METHODS

The Istituto Giannina Gaslini (IGG) is a paediatric hospital in northern Italy that acts as a local paediatric hospital for the town of Genoa and as a tertiary care hospital for other Italian

*Corresponding author
Elio Castagnola
E-mail: eliocastagnola@ospedale-gaslini.ge.it

regions and for patients from foreign countries. In 2007 a perspective surveillance program on alert pathogens and specific localized infections was activated according to the standards of the Joint Commission International.
At the end of 2011 the infection control team was under the impression of an increase in the incidence of Gram-negative UTIs and isolation of ESBL-producing or CAZ-R strains. Therefore an analysis of yearly epidemiology of these specific episodes was implemented for the period 2007-2011.
Episodes of UTIs, defined by the presence of 10^5 colony-forming units per millilitre (CFU7mL) of a single bacterial strain, were extracted from the Laboratory of Microbiology database [6]. In all cases urine sample collection was performed according to an internal, written procedure, specific to different age groups.
Pathogen identification, antibiotic susceptibility and ESBL production were performed by the automated Phoenix system (Becton Dickinson), even if automated systems may present some, well known pitfalls [7].
Susceptibility to antibiotics was evaluated using the Clinical and Laboratory Standards Institute (CLSI) recommendations from January 2007 to March 2011 [8]. From April 2011 the Microbiology Laboratory at the IGG modified its activity, using breakpoint tables drawn up by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to interpret MICs and zone diameters [9]. All isolated strains, both from inpatients and outpatients, were included without distinction between asymptomatic positive culture and clinically evident infection.
The epidemiology of Gram-negative UTIs and ESBL-producing and CAZ-R strains was analysed by calculating the rate of episodes, i.e. the number of UTIs observed in one year divided by the total number of hospitalization days in the same period, and expressed as episodes/1000 days of hospital admission. The epidemiology was evaluated considering all the UTIs observed at the IGG, and further focused on episodes observed in three specific units, Nephrology and Dialysis, Infectious Diseases, and Surgery, where urinary tract malformations are mostly treated.

Statistical analysis
The possible presence of a relation between the year of study and the rate of Gram-negative isolations in UTIs, detection of ESBL-producing strains and CAZ-R strains was analysed with the Pearson's correlation coefficient (r) that is a measure of linear association between two variables (10).
The correlation coefficient is measured on a scale that varies from + 1 through 0 to -1. Complete correlation between two variables is expressed by either + 1 or -1. When one variable increases as the other increases the correlation is positive; when one decreases as the other increases it is negative. Complete absence of correlation is represented by 0. The strength of possible association between the two variables is considered on the basis of absolute values of r: 0-0.19 (very weak), 0.2-0.39 (weak), 0.40-0.59 (moderate), 0.6-0.79 strong, 0.8-1 (very strong).
To test whether the association between year and isolation rates was merely apparent, and might have arisen by chance we used the *t* test in the following calculation:

$t = r\sqrt{[(\text{number of observations} - 2 \text{ degrees of freedom})/(1-r^2)]}$.

The *t* value was compared with specific tables of two-tailed distribution, and a $P \leq 0.05$ was considered as significant (10). Calculations of r and *t* coefficients were performed by means of Microsoft Excel 2011 for Macintosh (Microsoft Corporation 2010).

RESULTS

During the study period we observed a change in the rate of UTIs at the IGG, that overall increased from 2.69 in 2007 to 4.54 in 2011. The r coefficient for this change during the study period was 0.79, meaning a strong correlation between the two variables, but the P value was not statistically significant. As shown in table 1, which reports the rates of UTI due to Gram-negative and ESBL-producing or CAZ-R strains, in the same period, there were also changes in the rates of UTI diagnosed in the hospital units where children with UTI are routinely managed.
However, the changes in the Nephrology Unit had a coefficient r=0.95, showing a very strong correlation ($P < 0.05$), while the changes observed in the Infectious Diseases Unit had a strong correlation (r=0.77), but a non-significant P value, and that observed in the Surgery Unit had a weak correlation (r=0.38), without statistical significance.

Table 1 - Rates (episodes/1000 patient-days at risk) of Gram-negative urinary tract infections, isolation of ESBL-producing and ceftazidime-resistant strains.

	2007	*2008*	*2009*	*2010*	*2011*	*r coefficient*	*P value*
Urinary Tract Infections							
IGG	2.69	3.97	4.82	4.54	4.45	0.77	ns
Nephrology	11.63	10.85	19.62	26.28	27.48	0.95	<0.05
Infectious Diseases	1.81	1.86	1.87	2.83	4.45	0.77	ns
Surgery	2.04	3.13	5.14	3.26	3.32	0.65	ns
Extended Spectrum Beta-Lactamase-producers							
IGG	0.25	0.32	0.68	0.39	0.46	0.47	ns
Nephrology	0.54	0.8	1.31	1.13	2.55	0.89	0.05
Infectious Diseases	0.14	0.25	0.27	0.39	0.14	0.21	ns
Surgery	0.09	0.09	1.11	0.32	0.44	0.35	ns
Ceftazidime-resistant isolates							
IGG	2.98	2.86	3.88	3.16	3.68	0.60	ns
Nephrology	4.18	2.23	2.28	3.94	5.89	0.57	ns
Infectious Diseases	0.65	1.49	0.8	1.16	1.00	0.18	ns
Surgery	0.89	0.92	2.22	0.95	1.99	0.54	ns

Legend: IGG=Istituto Giannina Gaslini; ns = not significant.

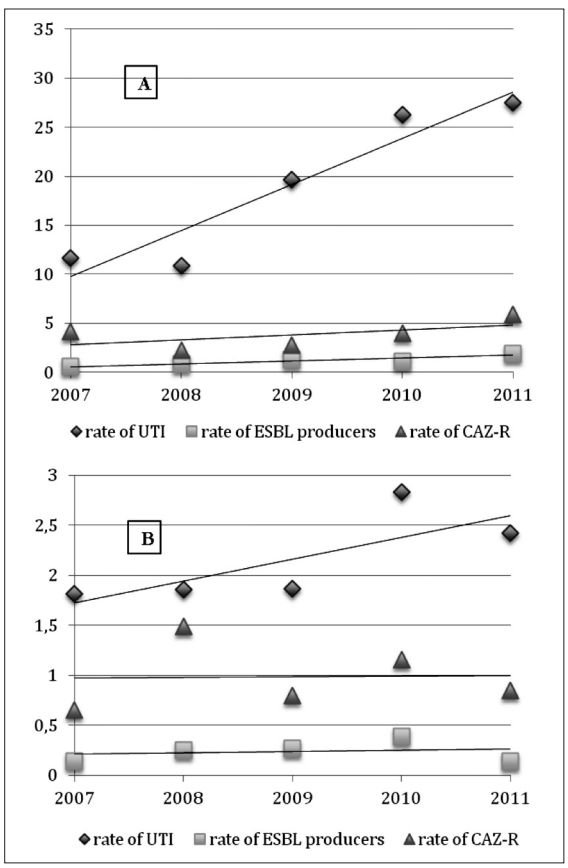

Figure 1 - Plots and regression line of rates of Gram-negative urinary tract infections and isolation of ESBL-producing isolates. A: Nephrology Unit, B: Infectious Diseases Unit.

As regards the isolation of resistant strains, we observed a very strong correlation between year and rate of ESBL-producing Gram-negatives only in the Nephrology Unit (r=0.89, P=0.05), a strong but not statistically significant correlation in the Infectious Diseases Unit, while the changes observed in the Surgery Unit and in the IGG overall were with moderate or weak correlation and without any statistical significance (Table 1).

Data regarding the rate of isolation of CAZ-R strains indicate an increase in infections due to these pathogens, albeit in the presence of a very weak or, at best, moderate correlation between year and rates of isolation in all the subgroups analysed (Tab. 1). To gain insights into the correlation between the year of study and the rates of UTI and isolation of ESBL-producing strains we created figure 1, which illustrates the data plotted together with the linear regression equation for the Nephrology (panel A) and Infectious Diseases (panel B) Units, i.e. those with strong or very strong correlations between year of study and rates of infection. No episode due to carbapenemase-producing isolates was observed.

DISCUSSION

UTI is one of the most common bacterial infections both in adults and in children and Gram-

negative bacteria are the most common aetiological agents involved. Moreover, in the paediatric population, in particular in early childhood, UTI is the most common cause of fever without any clinical sign of other sites of infection. The presence of vescicoureteral reflux is the most common risk factor related to UTIs, with other genito-urinary malformations and neurogenic bladder dysfunction being less frequently observed [11, 12].

From 2007 to 2011 we observed an increase in the rate of diagnosis of UTI due to Gram-negative at IGG. This variation was most notable in the Nephrology Unit which is the referral ward within the hospital, especially for patients with malformations and neurogenic bladder dysfunction.

In these patients we noted a very strong correlation between the year of observation and the increasing rate of UTI, with a significant P value, suggesting that this observation was not merely random.

The increase in UTI was also observed in the Infectious Diseases Unit, where children with acute febrile illness are frequently primarily admitted. In this case the correlation was not as strong as the previous one and lacked statistical significance. However, this does not strictly mean that the observation might have arisen by chance, but that perhaps more prospective data are needed to observe even statistical significance in a ward where UTIs are admitted non-electively [10].

Interestingly, in the Surgery Unit, where urinary tract malformations receive surgical treatment, changes in the rate of UTI diagnosis were not significant through the study period. This increase in UTIs was associated with a strong and significant increase in the rate of isolation of ESBL-producing Gram-negatives in the Nephrology Unit, but not in other wards. Also the rate of CAZ-R Gram-negative isolates increased in the Nephrology Unit, but not in a statistically significant fashion.

The increase in resistant pathogens as a result of UTIs has been observed primarily in adults [13-17]. Interestingly, in a prospective study conducted in the Calgary Health Region, 28% of ESBL *E.coli* infections were healthcare-acquired [18].

Our results clearly indicate that this phenomenon is also occurring in children, and especially in wards where administration of long-term, low-dosage antibiotics for prophylaxis of infections is common practice, especially in the presence of genito-urinary malformations that may represent a risk factor for the development of resistance among bacteria causing the infections [12, 19]. These data indicate the risk that in the near future antibiotic-resistant Gram-negative bacteria might represent a severe problem for children with genito-urinary malformations and UTIs, and the need for new, different prophylactic strategies in order to avoid the risk of selecting resistant bacteria that have no therapeutic options.

Keywords: urinary tract infections, paediatrics, extended spectrum beta-lactamase, ceftazidime resistance.

RIASSUNTO

Urinary tract infections (UTIs) are an important cause of morbidity in paediatrics, especially related to urinary tract malformation and neurogenic bladder dysfunction.

The infection control team of Istituto Giannina Gaslini, Genova, Italy, performs a prospective survey on the epidemiology of UTI in children admitted in the hospital, and data are expressed as episodes/1000 days of hospital admission.

From 2007 to 2011 there was an increase in the rate of Gram-negative UTIs, especially in the Nephrology Unit (from 11.63 to 27.48, r-coefficient 0.95, $P<0.05$), associated with an increase in infections due to ESBL-producing strains (from 0.54 to 2.55, r-coefficient 0.89, $P=0.05$). This study indicates that there is an increase in the rate of Gram-negative UTIs, also due to resistant strains.

The cause may be multifactorial, but it is noteworthy that it has been mainly observed in a ward where low-dose, long-term administration of antibacterial prophylaxis in children with urinary malformations or neurogenic bladder dysfunction is routine.

This phenomenon gives cause for concern and should be monitored carefully to avoid the risk of selecting resistant bacteria that have no therapeutic options.

SUMMARY

Le infezioni delle vie urinarie (IVU) sono una importante causa di morbosità in pediatria, specialmente in presenza di malformazioni delle vie urinarie o disfunzioni neurologiche della vescica. Il Comitato Infezioni Ospedaliere dell'Istituto Giannina Gaslini - Genova - esegue una sorveglianza prospettica sulla epidemiologia delle IVU in bambini ricoverati presso l'Istituto, e i dati sono riportati come tasso di episodi/1000 giornate di ricovero. Nel periodo 2007-2011 si è osservato un aumento dell'incidenza delle IVU da batteri Gram-negativi, soprattutto nell'Unità Operativa di Nefrologia (da 11.63 a 27.48, coefficiente r 0.95; P<0.05), associate ad un aumento del tasso di infezioni da ceppi ESBL-produttori (da 0.54 a 2.55, coefficiente r 0.89; P=0.05). Questo studio dimostra un aumento delle IVU da Gram-negativi, specie resistenti ai beta lattamici, in pediatria. Le cause possono essere multiple, ma è significativo che questa variazione epidemiologica si osservi soprattutto in un reparto dove viene routinariamente utilizzata profilassi antibatterica per tempi molto lunghi e a basso dosaggio. Questo fenomeno è preoccupante e necessita di monitoraggio attento al fine di ridurre il rischio di selezione di ceppi batterici con scarse o nulle opzioni terapeutiche.

REFERENCES

[1] Wald E.R. Cystitis and pyelonephritis. In: Feigin RD, Cherry JD, Demmler-Harrison G, Kaplan SL, eds. Feigin & Cherry's Textbook of pediatric infectious diseases. Philadelphia, PA: Saunders Elsevier. 554-68, 2009.

[2] Campobasso P. Antibiotic prophylaxys in pediatric surgery of genito-urinary abnormalities. *Infezioni in Medicina.* 2, 88-92, 2002.

[3] Edlund C., Nord C. Effect on the human normal microflora of oral antibiotics for treatment of urinary tract infections *J. Antimicrob. Chemother.* 46, 41-48, 2000.

[4] Meier S., Weber R., Zbinden R., Ruef C., Hasse B. Extended-spectrum β-lactamase-producing Gram-negative pathogens in community-acquired urinary tract infections: an increasing challenge for antimicrobial therapy. *Infection* 39, 333-340, 2011.

[5] Kao C.C., Liu M.F., Lin C.F. Antimicrobial susceptibility and multiplex PCR screening of AmpC genes from isolates of Enterobacter cloacae, Citrobacter freundii, and Serratia marcescens. *J. Microbiol. Immunol. Infect.* 43, 180-187, 2010.

[6] Bassetti M., Repetto E. Diagnostic and therapeutic management of Gram negative infections. *Infezioni in Medicina* 16 (Suppl. 2), 22-29, 2008.

[7] Diamante P., Camporese A. Evaluation of Vitek 2 performance for identifying extended spectrum beta-lactamases in Enterobacteriaceae "other than Escherichia coli, Proteus mirabilis and Klebsiella spp. *Infezioni in Medicina* 4, 216-226, 2006.

[8] (CLSI). CaLSI. Performance standards for antimicrobial susceptibility testing; 20th Informational Supplement (June 2010 Update) CLSI document M100-S20-U. Wayne, PA, USA: CLSI, 2010; 2010.

[9] Leclercq R., Cantón R., Brown D.F., et al. EUCAST expert rules in antimicrobial susceptibility testing. *Clin. Microbiol. Infect.* 19 (2), 141-160, 2013.

[10] Swinscow T.D.V., revised by Campbell MJ. Statistics at Square One. London: BMJ Publishing Group; 1997.

[11] Peters C.A., Skoog S.J., Arant B.S.J., et al. Summary of the AUA Guideline on Management of Primary Vesicoureteral Reflux in Children. *J. Urol.* 184, 1134-1144, 2010.

[12] Zegers B.S., SWinkler-Seinstra P.L., Uiterwaal C.S., et al. Urinary tract infections in children with spina bifida: an inventory of 41 European centers. *Pediatr. Nephrol.* 24, 783-388, 2009.

[13] Bellissima P, Amato R. Incidence of extended spectrum beta-lactamase (ESBL)-producing Enterobacteria in the area of Caltagirone (Sicily). *Infezioni in Medicina.* 2, 108-112, 2004.

[14] Gattuso G., Tomasoni D., Palvarini L., et al. Epidemiology of ESBL-positive Enterobacteriaceae in Mantova hospital (Italy). *Infezioni in Medicina.* 3, 164-168, 2009.

[15] Nicolle E. Update in adult urinary tract infection. *Curr. Infect. Dis. Rep.* 13, 552-560, 2011.

[16] Rodríguez-Baño J., Alcalá J.C., Cisneros J.M., et al. Community infections caused by extended-spectrum beta-lactamase-producing Escherichia coli. *Arch. Intern. Med.* 22, 168 (17), 1897-1902, 2008.

[17] Grandesso S., Sapino B., Mazzucato S., Alessandrini R., Solinas M., Gion M. Study on in-vitro susceptibility of ESBL-positive Escherichia coli isolated from urine specimens. *Infezioni in Medicina.* 3, 162-168, 2010.

[18] Laupland K.B., Church D.L., Vidakovich J., Mucenski M., Pitout J.D. Community-onset extended-spectrum lactamase (ESBL) producing E. coli: importance of international travel. *J. Infect.* 57, 441-448, 2008.

[19] Vijayakumar M., Kanitkar M., Nammalwar B.R., Bagga A., for the Indian Society of Pediatric Nephrology. Revised statement on management of urinary tract infections. *Indian. Pediatr.* 48, 709-717, 2011.