Infect Dis Trop Med 2017; 3 (4): e427

Susceptibility to anti-infective drugs of aerobic bacteria and fungi isolated from CASP: retrospective study in a pediatric tertiary care center

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ABSTRACT:

- ***Background:*** Perforated appendicitis is the most important cause of community acquired secondary peritonitis (CASP) in children. Knowledge of antimicrobial susceptibility of isolated pathogens is mandatory for a correct administration of empiric therapy.
- ***Patients and Methods:*** Retrospective analysis of pathogens isolated from peritoneal fluid in children with CAPS followed at the Istituto Giannina Gaslini (Genoa, Italy) from 2013 to 2015.
- ***Results:*** A total of 117 bacterial strains were detected: 52% *Enterobacteriaceae*, 14% *P. aeruginosa*, 19% Gram-positives, 13% Gram-negative anaerobes, and 2% Fungi. Resistance to ampicillin-sulbactam was detected in 33% of *Enterobacteriaceae* and 10% of *enterococci*. Moreover 15% of *Enterobacteriaceae* were resistant to gentamycin and 10% to piperacillin-tazobactam. No strain of *P. aeruginosa* was resistant to piperacillin-tazobactam. None of the Gram-negatives was resistant to cerbapenems and no Gram-positive strain was resistant to vancomycin. Concomitant resistance to ampicillin-sulbactam and gentamycin was detected in 13% of strains, while no strain was concomitantly resistant to piperacillin-tazobactam and gentamycin. These data were different from a previous 6-year observation in our centre.
- ***Conclusions:*** The present study demonstrates that continuous monitoring of aetiology and antimicrobial susceptibility is mandatory for a correct management of CASP in children.

- ***Keywords:*** CASP, Paediatric, Peritonitis, Drug-susceptibility, Piperacillin-tazobactam.

INTRODUCTION

Intra-abdominal infection (IAI) comprises a wide spectrum of pathological conditions, ranging from uncomplicated appendicitis to fecal peritonitis (intra-abdominal abscesses or diffuse peritonitis) (1). IAI may be classified according to whether the infection is community acquired (approximately 80%) or health care associated, the latter being the most common complication of elective or emergency intra-abdominal surgery. In children the most important causes of secondary peritonitis are perforated appendicitis and intra-abdominal abscess arising from acute appendicitis[1]. The etiology of this disease is predominantly related to the organisms of the gut flora such as *Enterobacteriaceae,* enteric Gram-positive *cocci*, and obligate anaerobes[2,3]. The exact role of *Pseudomonas aeruginosa* in this condition remains poorly clarified, even if it represents a non negligible proportions of pathogens isolated in pediatric secondary peritonitis[4]. Studies of antibiotic susceptibility of pathogens isolated in Community Acquired Secondary Peritonitis (CASP) in children are limited[1,5,6]. These issues are critical in an

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attempt to choose empirical, initial antibiotic treatment of secondary peritonitis. The most frequently suggested treatments are represented by monotherapy with a carbapenem or the combination of a penicillin with a beta-lactamase inhibitors like piperacillin-tazobactam or ticarcillin-clavulanate, or amoxicillin-clavulanate, ertapenem or piperacillin-tazobactam in absence of extended-spectrum-beta-lactamase (ESBL) producing strains. Meropenem or imipenem-cilastatin is recommended in case of suspicion of infections due to resistant strains[7,8]. In any case the choice of therapy, both for children and adults, should also be driven by severity of clinical condition, and/or local epidemiological data[8,9].

In 2013, a standard operative procedure for the initial empirical treatment of CASP was established in our hospital[10]. The choice of antibiotics was based on antimicrobial susceptibility of pathogens isolated from CASP in the 6 previous years, and the combination of ampicillin-sulbactam and gentamycin was suggested. At the same time, a prospective survey on the number of re-admission to the surgical ward of patients previously undergoing surgery for CASP was implemented and showed a 13.5% of re-interventions compared with the 5.6% of the previous three years. Surgical procedures were reviewed as well as microbiology and antimicrobial susceptibility of peritoneal isolated after 2013 in order to update the recommendations. The aim of the present study is to report changes in antimicrobial susceptibility of aerobic bacteria isolated from peritoneal cultures in children with CASP in the period January 2013 – December 2015.

PATIENTS AND METHODS

Patients

Istituto Giannina Gaslini (IGG), Genoa - Italy is a Tertiary Care Pediatric Hospital in Northern Italy serving as local Pediatric Hospital for the Genoa area, and as tertiary care referring hospital for Italy and for many foreign countries. Clinical records of patients diagnosed with community acquired secondary peritonitis from January 2013 to December 2015 were retrospectively reviewed in order to collect data on pathogens isolated from the peritoneal fluid and their susceptibility to anti-infective drugs. The diagnosis of CASP was based on 1) attending surgeon judgment based on the presence of transmural necrosis of the appendix, a defect in the wall of the appendix, an abscess cavity, or diffuse purulent fluid at the time of operation and 2) the absence of any risk factor (e.g. immunocompromission, inflammatory bowel disease, peritoneal dialysis, recent abdominal surgery), as reported on patient's clinical record.

Methods

Cultures were performed on peritoneal fluid sampled immediately after peritoneal incision. The peritoneal fluid was inoculated into both aerobic and anaerobic bottles (BactAlert Biomerieux, France) in the operating theatre, transferred to the laboratory within 1 hour after collection and incubated at 35°C under continued automated monitoring for seven days (BactAlert Detection System). Positive samples were Gram-stained and subcultured on agar plates according to laboratory standard methods[11]. Bacteria identification and antibiotic susceptibility tests (AST) were performed by automatic system (BD Diagnostics Systems, Franklin Lakes, NJ, USA) according to the manufacturer's recommendations. The ASTs were expressed as minimal inhibitory concentrations (MICs) and interpreted using EUCAST breakpoint tables[12]. Amoxicillin-clavulanate was substituted with ampicillin-sulbactam since only this drug was available for intravenous therapy in our Institute. AST for anaerobes was not evaluated. Antifungal susceptibility was evaluated by means of the YeastOne Sensititre (Trek Diagnostic System Ltd. West Sussex, UK).

RESULTS

During the study period, 117 strains were retrieved from cultures of peritoneal fluid in 76 patients with CASP. The patients median age was 7.8 years (range: 3-12 years), 40 were male (52%), while 36 (48%) were female. The majority of the patients were Italians (60; 79%) while 16 were foreign-born children (16; 21%). Cultures resulted polymicrobial in 42 (55%) cases

Table 1. Microorganisms Recovered From Peritoneal Fluid in children with community-acquired secondary peritonitis.

	Microorganisms	Number of strains (%)
Aerobes	*Gram-negatives*	77 (66)
	E. coli	53 (45)
	P. aeruginosa	16 (14)
	Klebsiella-Enterobacter-Serratia group	7 (6)
	P. mirabilis	1 (<1)
	Gram-positives	23 (19)
	Streptococci (S.constellatus, S.anginosus)	13 (11)
	E.faecalis	2 (2)
	E.faecium	5 (4)
	Enterococcus sp	3 (2)
Anaerobes	*Bacteroides spp*	15 (13)
Fungi		2 (2)
Total		117 (100)

and monomicrobial in 34 (45%). Table 1 reports the proportions of different isolated pathogens. The most frequently isolated aerobe was *Escherichia coli* (45%), while *Pseudomonas aeruginosa* represented 14% of the identified strains. *Enterococci* represented 8% of the identified strains, Gram-negative anaerobes accounted for 13% of the identified strains, while 2% were *Candida albicans*.

Table 2 reports on antibiotic susceptibility of pathogens isolated from peritoneal cultures. Resistance to ampicillin-sulbactam was detected in 33% of tested strains, and considering the absence of activity of this drug against *Pseudomonas aeruginosa*, the total of resistant strains to ampicillin-sulbactam was 44 out of 100 aerobe strains identified. Moreover, in a further 27% (n=14) of tested strains the MIC was at the breakpoint. Resistance to gentamycin was detected in 9% of Gram-negative aerobes, but in another 7% (n=6) of the cases the MIC was at the breakpoint. As for piperacillin-tazobactam 91% of strain tested resulted susceptible. It is noteworthy that the highest proportion of resistant strains (29%) was found in the *Klebsiella-Enterobacter-Serratia* group. None of the tested strains was resistant to meropenem, with all MIC < 2 mg/L. Similar results were observed for ertapenem that was effective against all the *Enterobacteriaceae*, in all cases with MIC < 0.5 mg/L. Finally, none of the enterococci was gentamycin or vancomycin-resistant, but 1 (10% of enterococci) strain was resistant to ampicillin.

Concomitant resistance to ampicillin-sulbactam and gentamycin was detected in 14% of *Enterobacteriaceae* (n=7), while no strain was concomitantly resistant to piperacillin-tazobactam and gentamycin. As for fungal pathogens, both strains of *Candida albicans* isolated from peritoneal fluid were resistant to fluconazole, but susceptible to amphotericin B and micafungin. No data for antibiotic susceptibility of anaerobes were available.

DISCUSSION

Perforated appendicitis and intra-abdominal abscess arising from acute appendicitis represent the most important cause of CASP in children. In our series, *Escherichia coli* and *Pseudomonas aeruginosa* followed by *Bacteroides* were the most frequently isolated organisms involved in CASP, even if these proportions were different from those observed in previous pediatric studies, as summarized in Table 3[6,10,13-16]. These differences might be attributable, at least in part, to differences among the populations evaluated in in previous studies, since our patients were affected only by appendicitis-induced peritonitis, while in some other reports were considered all patients with intra-abdominal infections requiring surgery. Knowledge of the bacterial susceptibility to antibiotics is critical in an attempt to provide the best antibiotic treatment since the beginning. The more recent recommendations have suggested monotherapy with ampicillin-sulbactam or ertapenem or piperacillin-tazobactam for primary empirical treatment in absence of risk factors for infections by resistant pathogens[7-9].

Table 2. Distribution of susceptibility to different antibiotics in children with advanced peritonitis.

Microorganism (n=number of tested strains)	Minimal Inhibitory Concentration (mg/L) and interpretation (n=number of strains)*	*E. coli* n=53**	*P. aeruginosa* n=16	*Klebsiella-Enterobacter-Serratia* group n=7**	*Proteus spp.* n=1**	*E. faecalis* n=2**	*E. faecium* n=5**	*Enterococcus spp.* n=3 (**)	*Streptococci* n=13**
Ampicillin-sulbactam (n= 84)	≤8 (S) n=56 (67) >8 (R) n=28 (33)	30 (57) 23 (43)	— —	3 (43) 4 (57)	1 (100) —	2 (100) —	4 (80) 1 (20)	3 (100) —	13 (100) —
Gentamicin (n=87)	≤4 (S) n=79 (91) >4 (R) n=8 (9)	45 (85) 8 (15)	16 (100) —	7 (100) —	1 (100) —	2 (100) —	5 (100) —	3 (100) —	— —
Piperacillin-tazobactam (n=77)	≤16 (S) n=70 (91) >16 (R) n=7 (9)	48 (90) 5 (10)	16 (100) —	5 (71) 2 (29)	1 (100) —	— —	— —	— —	— —
Meropenem (n=77)	< 8 (S) n=77 (100) >8 (R) n=0	53 (100) —	16 (100) —	7 (100) —	1 (100) —	— —	— —	— —	— —
Ertapenem (n=54)	≤ 1 (S) n=54 (100) >1 (R) n=0	53 (100) —	— —	— —	1 (100) —	— —	— —	— —	— —
Vancomycin (n=10)	≤4 S n=10 (100) >4 (R) n=0	— —	— —	— —	— —	2 (100) —	5 (100) —	3 (100) —	— —

*Numbers in parenthesis are proportions of the total number of tested strains.
** Numbers in parenthesis are proportions of the total number of isolated strains.

Table 3. Proportions of bacteria isolated from CASP in children.

Study/Year of publication (Reference)	Present study	Castagnola E et al[10]	Guillet-Caruba et al[15]	Dumont et al[16]	Lin et al[6]	Bennion* et al[14]	Brook et al[13]
Number of patients	76	106	86	70	100	30	113
Number of strains	117	114	136	123	445	305	325
Proportions of isolated bacteria							
E. coli	45	74	71	51	31	82	13
Other *Enterobactericeae*	6	8	7	4	11	10	4.7
P. aeruginosa	14	13	19	6	6	23	2
Bacteroides spp	13	—	20	22	9	73	23
Streptococci	11	—	34	9	18	33	12
Enterococci	8	6	8	1	3	33	2.6

*all polymicrobial infection.

In our previous study we documented that these recommendations could be not suitable for children or at least in all pediatric centers[10]. In the present study, we also showed that continuous evaluation of epidemiology and antimicrobial susceptibility is mandatory, since the combination ampicillin-sulbactam + gentamycin that in the period 2005-2011 had a potential 99% effectiveness, 2 years later was effective only in 86% of isolated strains (-13%)[10]. One of the most important statements of the guidelines for the treatment of peritoneal infections is that "if resistance to a given antibiotic is present in 10-20% or more of isolates of a common intra-abdominal pathogen in the community, use of that agent should be avoided[7-9]". According to this advice, we decided to change from the combination with ampicillin-sulbactam and gentamycin to piperacillin-tazobactam and gentamycin that allowed a potential effectiveness of 99-100%[17]. This decision was also made taking into account the high proportion of *Pseudomonas aeruginosa* we observed in the present study and in our previous survey[10].

CONCLUSIONS

The high proportion of *Pseudomonas aeruginosa* isolated in peritonitis is a peculiarity of pediatric CASP and must not be neglected[7,18-21]. The use of piperacillin-tazobactam and gentamycin is correct also taking in consideration the antibiotic susceptibility of other pathogens like *enterococci* (10% resistance to ampicillin, 0% resistance to gentamycin) and *streptococci* (100% sensitivity to ampicillin). Even if not evaluated, piperacillin is effective also against anaerobes giving another reason for choosing it as a CASP first-line empiric treatment.

Conflicts of Interest:
The authors declare no conflict of interest.

Statement of financial support:
No financial support

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