Trend of antibiotic resistance in 1316 *Shigella* strains isolated in Bahrain

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It is estimated that *Shigella* is to blame worldwide for 600,000 deaths per year, 60% of which occur in children. Investigating the evolutionary relationships of *Shigella*, it was found[1] that they are within the *Escherichia coli* (*E coli*), not representing a genus by them. Probably they evolved within the last 35,000 - 270,000 years, one of the early infections afflicting humans. *Shigella sonnei* (*S. sonnei*) and *Shigella flexneri* (*S. flexneri*) are the most common isolated organisms but *Shigella dysenteriae* (*S. dysenteriae*) is responsible for the most severe cases. *Shigellosis*, differently from other diarrheal diseases for which fluid replacement therapy can be usually considered satisfactory, represents a major indication for antimicrobial therapy. All over the world reports of multiple drug resistance are well documented,[2] as well as in the Middle East.[3,4] Although percentages vary in the different studies from various areas, reflecting different epidemiology and antibiotic usage, comparisons over subsequent years show a consistent increase in resistance over time.

We report our experience on antibiotic susceptibilities during the period 1994-2001 on a total of 1316 strains all isolated from stools, comparing the obtained results with relevant data gathered in the period 1984-1988. Stool specimens were inoculated on deoxycholate agar (DCA), selenite broth, subcultured on DCA and incubated at 37°C for 24 hours. Suspected colonies were further biochemically tested and serogrouped by the slide agglutination test (Murex Biotech LTD, England). In vitro, antibiotic sensitivity testing was performed by the standard disc (Mast, England) diffusion method. Interpretation criteria were according to the National Committee for Clinical Laboratory Standards (NCCLS) manual. The quality control organism was *E coli* american type culture collection 25922. The highest number of isolates was observed for *S. sonnei* (664 strains; 50.5%), followed by *S. flexneri* (450 strains; 34.2%). The lowest number of isolates was detected for *Shigella boydii* (*S. boydii*) (122; 9.3%,) presenting a peak in 1994, and *S. dysenteriae* (80 isolates; 6.1%), with a disquieting peak in 2000. The isolation rate for the different species did not change over a long period of time. A previous investigation from our Laboratory[4] covering the period 1984-1988 showed *S. sonnei* most abundant (48%), followed by *S. flexneri* (40%), *S. boydii* (8.4%), and *Shigella dysenteriae* (3%). Thus, the relative incidence of various species is constant during a long periods. Isolation rates were higher in children (below 15 years of age) showing modest variations during the period investigated (70.8-74.8%). No increasing trend in the number of cases was noted. From **Table 1**, it is evident that in the last 3 years, stable values for resistance to chloraphenicol (CAF), co-trimoxazole (SXT) and ampicillin (AMP), were recorded. More relevant is the dramatic increase (but it can represent, a worrying peak,) in resistance to Cefuroxime. These data was obtained from 108 strains tested and not from all isolates. This antibiotic was tested as it is widely used by pediatricians, who cannot rely on ciprofloxacin, as it is not approved for use in children. From **Table 2**, where data was arranged for different subgroups, it is apparent that resistance to the various antibiotics tested varies according to the different species. *S. sonnei* shows the uppermost resistance to SXT (range 77.2-98%) similar to what was observed for *S. flexneri.* All strains were found sensitive to ciprofloxacin and ceftriaxone. Sensitivity to cefotaxime and to ceftriaxone indicates that the plasmid described coding for an extended spectrum beta-lactamase (ESBL) is not yet present in our region. Resistance to cefuroxime (a surprising peak of 20% in 2000 for *S. boydii* and of 63.6% for *S. dysenteriae* in 2001) is a very alarming and needs to be investigated further. However, the most striking result is the trend of resistance over the years for the 2 most commonly isolated subgroups. Regarding *S. sonnei*, no increasing trend in resistance was found: AMP is at its lowest in 2000 with values in 2001 lower than those observed in 1994 and SXT appears constant at a very high percentage. The very low CAF resistance rate is equal to what it was 8 years ago. In *S. flexneri* resistance to AMP is significantly declining, from the peak observed in 1996 (95%) down to 54.7% in 2000, showing a further significant decrease to 52.6% in 1999. A similar trend is shown for SXT, decrease from 83.4% in 1994 to 52.8% in 2000. A similar pattern is also observed for CAF, now on a value of 44.4% compared to the peak of 90% in 1996 and the average of 78.5 % over the period 1995-1998. Intriguingly enough, this trend is not to be observed with other subgroups where the number of resistant strains isolated was erratic during the years. The only increasing trend is related to resistance to cefuroxime for *S. flexneri*, which can only represent an isolated peak rather than a consistent climb. Comparing the percentage of antibiotic resistant strains tested in the year 1987-1988[13] and 2000, it is evident that different species behave in different ways. However, a trend for decrease or no change was observed for *S. flexneri* and *S. boydii*, while for *S. sonnei* a minor increase was found for CAF resistance while a decrease was detected for ampicillin. The only species showing a uniform tendency to increased resistance was *S. dysenteriae*, the least common isolate. We can conclude that the profile of resistance is variable in the diverse species and that antibiotic resistance in all *Shigella* subgroups, at least in our region, is not on the

Table 1 - *Shigella* species strains (%) resistant to the tested antibiotics (N = 1,316).

Antibiotics	2001 N = 118	2000 N = 195	1999 N = 121	1997 N = 186	1996 N = 219	1995 N = 103	1994 N = 204
Ampicillin	38.4	32.3	24.8	52.2	36	39	51.5
Ceftriaxone	0	0	0	0	0	0	0
Cefuroxime	11.1*	26.2	NT	0.5	6	0	1.5
Cefotaxime	0	0	0	NT	NT	NT	NT
Co-trimoxazole	59.3	65.5	66.1	89.3	87	76.7	72
Chloramphenicol	27.1	28.8	19.8	32.3	28	30	35.3
Ciprofloxacin	0	0	0	0	0	0	0

*only 108 strains have been tested, NT - not tested

Table 2 - Resistance of different *Shigella* species to routinely tested antibiotics (N=1,316).

Strains resistant to the tested antibiotics	Ampicillin %	Ceftriaxone %	Cefuroxime %	Cefotaxime %	Co-trimoxazole %	Chloramphenicol %	Ciprofloxacin %
S. sonnei (N = 664)							
2001 n = 44	11.3	0	1.3	0	77.2	4.5	0
2000 n = 98	5.1	0	1.4	0	85.7	3.7	0
1999 n = 67	8.9	0	NT	0	80.6	0	0
1998 n = 97	6.2	0	0	0	88.6	2.1	0
1997 n = 103	28.1	0	0	0	94.2	0.97	0
1996 n = 135	6	0	2	NT	98	2	0
1995 n = 51	5.9	0	0	NT	88.2	3.9	0
1994 n = 69	14.5	0	0	NT	88.4	4.3	0
S. flexneri (N = 450)							
2001 n = 47	55.3	0	4.8	0	63.8	44.7	0
2000 n = 53	54.7	0	16.6	0	52.8	38	0
1999 n = 38	52.6	0	NT	0	60.5	57.9	0
1998 n = 60	68.3	0	0	3.3	75	76.6	0
1997 n = 72	83.3	0	1.4	NT	82	76.4	0
1996 n = 58	95	0	2	NT	81	90	0
1995 n = 38	76.3	0	0	NT	78.9	71	0
1994 n = 84	80	0	1.2	NT	83.4	66.6	0
S. boydii (N = 122)							
2001 n = 14	42.8	0	0	0	28.6	21.4	0
2000 n = 21	61.9	0	20	0	23.8	0	0
1999 n = 9	33.3	0	NT	0	22.2	22.2	0
1998 n = 9	33.3	0	0	0	44.4	33.3	0
1997 n = 8	62.5	0	0	NT	87.5	25	0
1996 n = 16	62	0	25	NT	44	19	0
1995 n = 7	57	0	0	NT	28.6	0	0
1994 n = 38	50	0	0	NT	31.6	23.6	0
S. dysenteriae (N = 80)							
2001 n = 13	69.2	0	63.6	0	15.4	61.5	0
2000 n = 23	59.6	0	5.5	0	47.8	71.4	0
1999 n = 7	14.3	0	NT	0	14.3	0	0
1998 n = 4	75	0	NT	0	50	0	0
1997 n = 3	100	0	0	NT	100	66.6	0
1996 n = 10	40	0	10	NT	40	30	0
1995 n = 7	57	0	0	NT	28.6	28	0
1994 n = 13	69.2	0	5.4	NT	30.8	30.8	0

S - *Shigella*, NT - not tested

rise but shows wide fluctuations over the years recorded. Our data, in view of the long period examined, can be compared with the data collected at the International Center for Diarrheal Disease Research in Bangladesh (ICDDR B).[5] In respect to the AMP report from ICDDR B, it shows an increase in resistance up to 90% in 1988 for *S. dysenteriae*, while in our experience a maximum of 100% was obtained in 1997 with a decrease to 60% in 2000. Considering all *Shigella species*, our current resistant rate is equal to 38.4%, similar to the values reported by ICDDR B in 1985. Comparing the current data with a previous study that was carried on from our institution, rates for AMP were unchanged; for *S. sonnei* 9.2% in 1987-1988 versus 8.2% in 2000-2001, for *S. boydii* 61% in 1987-1988 versus 52.3% in 2000-2001, and for *S. dysenteriae* 58% in 1987-1988 versus 64.4% in 2000-2001. A significant reduction from 75.6% in 1987-1988 to 55% in 2000-2001 was detected for *S. flexneri*. The data from ICDDR B for cotrimoxazole show that an increase was observed for all *Shigella* groups, from 20% in 1983 to 60% in 1991. Our data show a decrease from the peak was observed in 1997 (89.3%) to the current values, approximately 60%. A composite picture emerges from the comparison of present results with previous ones at our institution in the years 1987-88. While for *S. sonnei* there is an increase when compared with 2000-2001 (24.5-81.4%), an opposite trend has been detected for *S. boydii* and *S. dysenteriae* (with a decrease from 51% to 26.2% and from 40% to 31.6%). It is reported that, particularly in developing countries, quinolone resistance is on the rise.[5] We did not isolate a single strain resistant to ciprofloxacin, indicating that factors favoring the appearance of the chromosomal mutation involved in this type of resistance, are not operating in our population. More worrisome is the TEM-1 beta-lactamase mediated increase in resistance to cefuroxime, most likely associated with plasmid-encoded beta-lactamase production.[5] Interestingly ceftriaxone and cefotaxime retained their activity on all isolates. From this long term analysis it seems that when, for diverse reasons, antibiotic selective pressure is reduced, bacteria can revert to sensitivity. This propensity can be exploited in a rational and planned way to make again efficacious old, safe and less costly antibiotics.

Acknowledgment. This study was supported by a Research Grant from Arabian Gulf University to Prof. Giuseppe A. Botta. Thanks also to Tomy Kaitharath for his skillful typing assistance.

Received 30th September 2002. Accepted for publication in final form 13th October 2002.

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