LABORATORY SURVEILLANCE OF *SHIGELLA DYSENTERIAE* TYPE 1 IN KWAZULU-NATAL

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Objective. To collect data on the antimicrobial susceptibility of *Shigella dysenteriae* type 1 in KwaZulu-Natal, including the testing of newer therapeutic agents, and to evaluate the ability of laboratories to participate in a provincial surveillance programme.

Design. Prospective descriptive study.

Setting. Hospital laboratories in KwaZulu-Natal, including peripheral laboratories and the medical microbiology laboratory of the University of Natal.

Main outcome measures. Antimicrobial susceptibility pattern of surveillance strains and evaluation of the ability of provincial laboratories to isolate *Shigella*.

Results. All 354 strains tested were resistant to ampicillin, chloramphenicol and tetracycline. Co-trimoxazole resistance was found in 99.2% of strains, and 0.8% of strains were resistant to nalidixic acid. All strains were susceptible to ceftriaxone, ciprofloxacin, ofloxacin, pivmecillinam, azithromycin, loracarbef and fosfomycin. Of the 29 laboratories surveyed, 18 (62.1%) were able to isolate and identify *S. dysenteriae* correctly, and 9 (32%) were able to serotype it further to *S. dysenteriae* type 1. Twenty-seven (93.1%) had appropriate culture media and 26 (89.7%) had antisera for *Shigella* identification.

Conclusions. There is little variation among strains of *S. dysenteriae* type 1 in KwaZulu-Natal with regard to their antimicrobial susceptibility pattern. Nalidixic acid should remain the antimicrobial of choice for treatment of dysentery in our region as resistance to it is low. The majority of KwaZulu-Natal laboratories have the expertise and equipment to perform the isolation and identification of *Shigella* species.

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An outbreak of *Shigella dysenteriae* type 1 infection has been ongoing in KwaZulu-Natal since the beginning of 1994.[1-3] This pathogen, which is the major cause of epidemic dysentery in Africa, appears to have spread southwards to South Africa from other parts of Africa, namely Zaire, Burundi, Zambia, Zimbabwe and Mozambique, where large epidemics have been reported since the 1980s.[4,5] *S. dysenteriae* type 1 causes a spectrum of disease ranging from mild watery diarrhoea to dysentery, haemorrhagic colitis and toxic megacolon, and is associated with haemolytic uraemic syndrome (HUS).[6] The severity of infection of this serotype as opposed to other *Shigella* is thought to be due to the production of a potent neurocyto-enterotoxin known as Shiga toxin.[7] It is also multiply resistant to antibiotics such as ampicillin, co-trimoxazole, tetracycline and chloramphenicol. Of particular concern is the emerging resistance to quinolone antimicrobials such as nalidixic acid, with resistance as high as 57% in Burundi in 1994.[8]

Following the isolation of a nalidixic acid-resistant *S. dysenteriae* type 1 strain in KwaZulu-Natal[9] we decided to undertake a laboratory surveillance programme in order to determine the susceptibility pattern of the organism in our province. *S. dysenteriae* type 1 is not an organism that is easy to isolate from stools, and many epidemics in Africa have been misidentified as being of amoebic or viral causation.[4] We therefore also conducted a quality-control survey of laboratories in the province to determine their ability to culture *S. dysenteriae* type 1.

Material and methods

An instruction was issued in December 1994 through the provincial laboratory services of KwaZulu-Natal to all laboratories in the province to send isolates of *S. dysenteriae* type 1 to the medical microbiology laboratory of the University of Natal, Durban. Transport instructions were that they were to be sent on nutrient or equivalent agar slopes via routine transport channels. All isolates were stored on these slopes at 4 - 8°C until further testing. Data were recorded on the date of isolation, and the laboratory that had done the isolation was also recorded.

Stored isolates were sub-cultured onto xylose/lysine/deoxycholate agar (Oxoid, UK) and were confirmed as being *S. dysenteriae* type 1 by a negative catalase test, biochemical tests (glucose, mannitol, indole) and serotyping with type 1-specific antisera (Sanofi-Pasteur, France). Susceptibility testing was performed using the Kirby-Bauer disc diffusion method (National Committee for Clinical Laboratory Standards, Villanova, USA) for the following antibiotics and disc strengths: ampicillin 10 µg, co-trimoxazole 15 µg, tetracycline 30 µg, chloramphenicol 30 µg, nalidixic acid 30 µg, ciprofloxacin 5 µg, ofloxacin 5 µg, ceftriaxone 30 µg, azithromycin 15 µg, fosfomycin 200 µg, pivmecillinam 10 µg and loracarbef 30 µg. Extended-spectrum β-lactamase testing was performed using a double disc diffusion method.[10] During the study period a selection of consecutive isolates from King Edward VIII Hospital were included for susceptibility testing.

A quality-control survey was conducted on a random selection of laboratories to determine their ability to culture stool samples for *S. dysenteriae* type 1 and *Shigella flexneri*. Three freeze-dried quality-control specimens were prepared containing equal volumes of a McFarland 1.0 standard suspension of *Proteus mirabilis* and non-pathogenic *Escherichia coli*. The first specimen also had *S. dysenteriae* type 1, the second *S. flexneri* and the third no pathogen. Laboratories were requested to isolate and perform antibiotic susceptibility testing on clinically significant organisms. The following antibiotic susceptibilities were to be determined: ampicillin, tetracycline, chloramphenicol, co-trimoxazole, cefuroxime, cefotaxime, co-amoxiclav, gentamicin, nalidixic acid and ciprofloxacin. An error in susceptibility was defined as reporting a resistant strain susceptible, or reporting a susceptible strain resistant.

All data were captured and analysed using EpiInfo version 6 software (Centers for Disease Control, USA).

Results

A total of 521 isolates on nutrient agar slopes and sealed agar plates of varying quality were received. Of these 322 (61.8%) were confirmed to be *S. dysenteriae* type 1. Of the remaining cultures 5 (0.9%) yielded *S. flexneri*, 40 (7.8%) other Enterobacteriaceae, and 2 (0.4%) Gram-positive organisms; 152 (29.2%) had no growth.

The source of *S. dysenteriae* type 1 isolates is depicted in Fig. 1. Thirty-five of the isolates received were unlabelled as to their origin. For the susceptibility testing we included 32 isolates that had been isolated in our laboratory from patients presenting at King Edward VIII Hospital. The susceptibility pattern was determined in all 354 of the isolates, and was similar with only 6 exceptions (Table I). Three strains (0.8%),

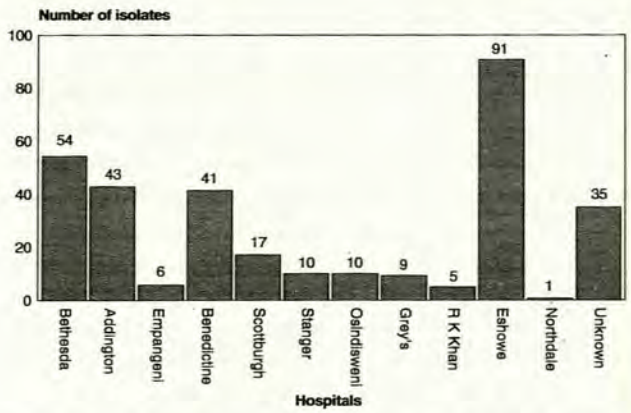

Fig. 1. Source of isolates — S. dysenteriae *type 1 isolates received from hospital laboratories in KwaZulu-Natal (N = 322).*

Table I. Antibiotic susceptibility of *S. dysenteriae* type 1 strains collected over the study period (*N* = 354)

Antibiotic	Susceptible	
	No.	%
Ampicillin	0	0
Co-trimoxazole	3	0.8
Tetracycline	0	0
Chloramphenicol	0	0
Nalidixic acid	351	99
Ciprofloxacin	354	100
Ofloxacin	354	100
Pivmecillinam	354	100
Ceftriaxone	354	100
Azithromycin	354	100
Fosfomycin	354	100
Loracarbef	354	100

1 each from Empangeni, King Edward VIII and Addington hospitals, were found to be resistant to nalidixic acid. Three isolates were found to be susceptible to co-trimoxazole, 2 from King Edward VIII and 1 from Eshowe Hospital. All isolates were found to be resistant to ampicillin, tetracycline and chloramphenicol, and all isolates were found to be susceptible to ceftriaxone, ciprofloxacin, ofloxacin, pivmecillinam, azithromycin, loracarbef and fosfomycin. Extended-spectrum β-lactamase production was not detected in any of the isolates.

Of the 29 laboratories surveyed, 23 (79%) were able to isolate and identify *Shigella* to the genus level, and 18 (62.1%) and 21 (72.4%) were able to identify *S. dysenteriae* and *S. flexneri* to the species level respectively. The full identification to serotype of *S. dysenteriae* type 1 was made by 9 laboratories (31%). Non-pathogenic species were reported as pathogens in 13 (44.8%) *S. dysenteriae* type 1 samples, 12 (41.3%) *S. flexneri* samples and 14 (48.3%) pathogen-free samples. A summary of the susceptibility results is shown in Table II. Analysis was difficul as only 2 of the laboratories had all the available discs. There were no errors for these 2 laboratories. XLD medium was available in 24 laboratories (82.8%), and DCA medium in 4 (13.7%). One laboratory had both of these media and 2 laboratories had neither. In addition 5 laboratories (17.2%) use Salmonella-Shigella medium. This included the 2 laboratories that had neither XLD nor DCA medium. Antisera for diagnosis of *Shigella* were available in 26 laboratories (89.7%).

Table II. Quality-control specimens — susceptibility testing of *S. dysenteriae* type 1 (*N* = 18)

	Number of laboratories with:		
	Discs available	Error: susceptible called resistant	Error: resistant called susceptible
Ampicillin	18	N/A	1
Tetracycline	15	N/A	0
Chloramphenicol	17	N/A	0
Co-trimoxazole	16	N/A	0
Nalidixic acid	9	0	N/A
Ciprofloxacin	6	0	N/A
Cefuroxime	16	0	N/A
Cefotaxime	16	0	N/A
Gentamicin	16	0	N/A
Co-amoxiclav	16	9	N/A

Discussion

A large number of isolates were analysed over the study perio and the majority were confirmed to be *S. dysenteriae* type 1. Th large number of no-growths among the specimens received ca probably be attributed to lack of appropriate transport resources; isolates were not always timeously and correctly transported. Some isolates were sent under conditions that would not ensure survival of the organism, whereas the initial recommendations for transporting the specimens would have kept the isolates viable for prolonged periods.

Surveillance isolates were received from only 11 laboratories. Reasons for other laboratories not sending isolates despite being situated in the epidemic region could include failure to isolate the organism owing to use of incorrect media and lack of proper training in isolating pathogens from stool, lack of transport for isolates to our laboratory and lack of motivation.

Four of the laboratories from which isolates were received were able to isolate more than 30 strains over the study period. These laboratories were from different regions; some were isolated rural hospital laboratories such as Benedictine (Nongoma), Bethesda (Ubombo) and Eshowe, and one was a tertiary care institution, namely Addington Hospital (Durban). It seems, therefore, that individual factors influenced whether laboratories were able to isolate the organism and whether they would participate in a surveillance programme. Education of laboratory staff regarding their responsibility in playing a greater role in public health by detecting and monitoring outbreaks of infectious diseases needs to be addressed.

Nalidixic acid is a quinolone antimicrobial agent and is the mainstay of therapy for epidemic multidrug-resistant *Shigella* dysentery. Resistance to this drug has been detected in *S. dysenteriae* type 1 outbreaks in Africa[8] and Asia.[11] The low level of resistance detected in our study is consistent with that described in Zimbabwe.[12]

Although nalidixic acid resistance does not appear to be a problem at present this situation could change rapidly, as was the case in Burundi (A. Ries — unpublished data). The mechanism of the rapid dissemination of resistance to nalidixic acid is not clear. Nalidixic acid resistance is a consequence of a change in the *Shigella* topoisomerase IV target site that results from a chromosomal change of the gyrA gene.[13] Plasmid-mediated resistance, which may result in rapid spread of

resistance, has not been detected for nalidixic acid; however, a plasmid designated pYD1, conferring trimethoprim resistance, has been shown to increase the frequency of mutation to nalidixic acid resistance in recipient strains.[14] It is possible that the dissemination of this plasmid may contribute to the spread of nalidixic acid resistance by inducing mutations in different strains of *S. dysenteriae* type 1. It is essential that we continue to monitor susceptibility of *Shigella* to nalidixic acid and to other agents that are also being used in the therapy of *S. dysenteriae* type 1 infections, namely ceftriaxone and ciprofloxacin.

The search for new antibiotics to treat shigellosis is important in the wake of its rapidly emerging resistance to present agents.[15,16] Among the newer antibiotics we tested, azithromycin has already been used in a clinical trial[17] and was found to be as good as a fluoroquinolone. Others such as fosfomycin have been used in the treatment of cases of *E. coli* 0157 infection, such as in the recent epidemic in Japan (T. Takeda *et al.* — unpublished data) while loracarbef still needs to be evaluated. Extended spectrum β-lactamase production among Enterobacteriaceae exists in our hospital and throughout KwaZulu-Natal (unpublished data). Owing to the widespread use of ceftriaxone and cefotaxime for the treatment of patients with severe dysentery in KwaZulu-Natal, we were concerned that resistance to third-generation cephalosporins might emerge. However, we did not detect any extended spectrum β-lactamase production among the isolates. Unlike other developing countries, South Africa has been able to afford the use of third-generation cephalosporins for the treatment of dysentery. Selective pressure with heavy use of these antimicrobial agents may result in cephalosporin resistance in *S. dysenteriae* type 1, which is quite likely to emerge in this country.

The quality-control survey indicates that problems exist in the quality of stool cultures in KwaZulu-Natal as only 79% of laboratories were able to isolate *Shigella* from the samples. There was also a high rate of reporting of non-pathogens, which indicates lack of training as regards interpretation of stool cultures. Considering the high inoculum of *Shigella* in the quality control samples, none of the laboratories should have had a negative culture result, suggesting that there is room for improvement in a province where *Shigella* infections are endemic. XLD and DCA agar are considered to be the best isolation media for the isolation of *Shigella*. Only 2 laboratories did not have either XLD or DCA medium and close to 90% had appropriate antisera, indicating that the reasons for missing *Shigella* are most likely incorrect media preparation or storage (important factors for both XLD and DCA), or inability of technologists to recognise possible *Shigella* colonies. When analysing the results of the susceptibility testing it was obvious that there was a shortage of antibiotic discs for performing appropriate susceptibility testing, and that there was considerable variation between laboratories where antibiotics were tested. Susceptibility testing should be standardised so that results of different laboratories can be compared and antibiotics that are part of the essential drugs list can be included. It is most likely that susceptible strains are reported as being resistant for co-amoxiclav because of deterioration of discs due to clavulanic acid being hygroscopic.

In view of increasing budgetary constraints and the expertise required and costs involved in performing accurate stool cultures, it would seem that the best possible solution would be to perform stool cultures at certain sentinel sites only. These sentinel sites should have properly trained staff and be fully equipped, and could form part of an infectious diseases surveillance network. This would provide more accurate information than having sub-standard cultures performed at all hospital laboratories. The information obtained from such sentinel sites would be more useful for epidemic control, and would give clinicians more accurate information regarding antimicrobial susceptibility and the nature of the causative pathogen than the current system.

It is clear from this report that KwaZulu-Natal lacks an adequate public health laboratory programme to monitor and detect infectious diseases. It is likely that this is also the case in many of the other provinces. The cost that KwaZulu-Natal has paid during this epidemic is enormous. It has been estimated that for every dysentery case presenting at a hospital, there are between 27 and 54 cases in the community.[18] The KwaZulu-Natal *Shigella* epidemic control team's sentinel site surveillance data[19] show that there are approximately 500 cases of bloody diarrhoea a month at the 10 sentinel sites, which suggests that there could be as many as 15 000 cases of dysentery a month in the sentinel site areas alone. Hospital mortality is estimated to be between 5% and 13%.[2,3]

The *S. dysenteriae* type 1 outbreak in KwaZulu-Natal has been the largest dysentery outbreak in South Africa to date. It is apparent that the epidemic is now spreading to other provinces such as the Eastern Cape and Western Cape (personal communication — Dr S Oliver, Department of Medical Microbiology, University of Cape Town).

Laboratory services in our country are currently undergoing a reconstruction process and there is a need for a reference laboratory service that would lend itself to playing a greater role in public health.

We recommend that nalidixic acid remain the antibiotic of choice for bacillary dysentery in our region and that the restructuring of laboratory services include a national infectious disease surveillance system.

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References

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