ANTIBIOTIC RESISTANCE PATTERN OF VIBRIO CHOLERAE AND SHIGELLA CAUSING DIARRHOEA OUTBREAKS IN THE EASTERN AFRICA REGION: 1994-1996

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SUMMARY

Between March 1994 and December 1996, 1797 rectal swabs were transported to the AMREF laboratory from sites in six countries in the eastern Africa region: 1749 were cultured for Vibrio cholerae and 48 for Shigella/Salmonella. Culture, isolation, identification and antibiotic susceptibility testing were performed using standardized techniques. The isolates were categorized as sensitive or resistant based on standardized zones of inhibition. The rate of isolation of V. cholerae from rectal swabs increased progressively from less than 20% to more than 45% between 1994 and 1996. 80-100% of isolates of V. cholerae from Kenya and south Sudan, and 65-90% from Somalia were sensitive to tetracycline, although in 1995 isolates from Mogadishu showed only 44% sensitivity. All isolates from Tanzania and Rwanda were 100% resistant to tetracycline. In Kenya and Somalia, the percentage of isolates sensitive to chloramphenicol and cotrimoxazole reduced markedly from 85% in 1994 to <10% in 1996. 100% of isolates from Rwanda and Tanzania were resistant to chloramphenicol and cotrimoxazole while in south Sudan >70% of isolates were sensitive. Nalidixic acid and erythromycin retained >75% sensitivity in all areas. Shigella dysenteriae and Shigella flexneri were recovered from dysentery specimens in northern Kenya. Both species showed similar antibiotic sensitivity patterns and were sensitive only to nalidixic acid and furazolidone. Due to variations in resistance patterns within countries in the region, antibiotic sensitivity testing should be performed at the start of an outbreak, and antibiotic use should be restricted to severe cases of V. cholerae and Shigella infection.

INTRODUCTION

Cholera outbreaks were first reported in the eastern African region between the years 1836 and 1876, spreading along the transportation routes from Arabia. These outbreaks affected mainly the coastal areas, and occurred seasonally during the monsoons. Cholera cases associated with the seventh pandemic were reported in the eastern African region in 1971, spreading through Sudan, Ethiopia, Somalia and Uganda into Kenya and extending south into Tanzania. These outbreaks occurred during a period of severe drought with an increased movement of people in search of food and water. Since then, acute watery diarrhoea caused by Vibrio cholerae O1 biotype El Tor has become endemic through transmission by asymptomatic and convalescent carriers, with seasonal outbreaks in various parts of the region and major outbreaks occurring in conflict areas.

Shigella dysentery occurs both endemically and epidemically throughout the eastern Africa region. A major outbreak was reported in 1979, starting in the western part of the region (Zaire) and spreading south through Rwanda and Burundi to Tanzania. Outbreaks were reported in Tanzania in 1981 and in the Rwandan refugee population in eastern Zaire in 1984.

The African Medical and Research Foundation (AMREF) is a non-governmental organization (NGO) working to support health care delivery and development in regional eastern Africa. In 1994, the AMREF laboratory established the capability for bacteriological analysis to provide prompt diagnostic support to health workers, particularly in the remote areas of the region. This study is a retrospective analysis of 1797 specimens submitted to the AMREF laboratory for investigation of diarrhoea outbreaks from sites in six countries in the eastern Africa region between March 1994 and December 1996.

MATERIALS AND METHODS

All the samples received were rectal swabs collected into Cary-Blair transport medium. One thousand seven hundred and forty nine samples were taken from patients with acute watery diarrhoea and were cultured for Vibrio cholerae; 48 samples were taken from patients with dysentery and were cultured for Shigella/Salmonella species. Samples for V. cholerae were cultured in alkaline peptone water and subcultured on thiosulphate citrate bile salt (TCBS) agar. V. cholerae colonies were identified by sugar fermentation in Kligler iron agar (KIA), by the oxidase reaction, by motility testing and by serological typing. Biotyping was not performed. Samples for Shigella/Salmonella were cultured directly onto deoxycholate citrate agar (DC) or were enriched in selenite F broth overnight followed by subculture on DC. Suspected Shigella/Salmonella colonies were identified by sugar fermentation in KIA, by lack of urease production on urea agar and by serological typing; subtyping was not performed. Antimicrobial sensitivity testing was performed using the Kirby-Bauer technique.
The antibiotic sensitivity patterns for each country are shown in Figures 3-7. Eighty to one hundred per cent of the isolates from Kenya and south Sudan, and 65-90% of the isolates from Somalia were sensitive to tetracycline. In Tanzania and Rwanda, 100% of isolates were resistant to tetracycline. In Kenya and Somalia, the percentage of isolates sensitive to chloramphenicol and cotrimoxazole reduced markedly during the three year period, from 85% in 1994 to less than 10% in 1996. One hundred per cent of the isolates from Rwanda and Tanzania were resistant to chloramphenicol and cotrimoxazole; but >70% of isolates from south Sudan were sensitive to these antibiotics. Ampicillin and furazolidone showed poor activity against V. cholerae isolates throughout the region. Nalidixic acid and erythromycin retained >75% sensitivity in all areas.

Bauer agar diffusion method using recommended antimicrobial agents at standard concentrations(6). Isolates were categorized as sensitive or resistant based on standardised zones of inhibition.

RESULTS

The areas from which isolates of V. cholerae and Shigella sp. were obtained are shown on the map(Figure 1). No information on the exact source of the samples, or data on the extent of each outbreak or mortality rates are available. Due to lack of information provided to the laboratory, the age and sex of the patients from whom isolates were obtained is not known.

Figure 1
East African region sites of outbreaks of diarrhoea investigated by AMREF

Cholera outbreaks: A total of 612 (35%) isolates of V. cholerae were cultured from 1749 rectal swabs received from Kenya, Tanzania, Somalia, Rwanda, and south Sudan. In Kenya and Somalia, where specimens were received every year for three years, the isolation rate increased from 18% (Kenya) and 16% (Somalia) in 1994 to 46% in 1996. The isolation rates from all five countries is shown in Figure 2. Five hundred and fifty two of the isolates were serotype Ogawa; 60 were serotype Inaba.

Figure 2
Isolation of V. cholerae from rectal swabs
Shigella outbreaks: Thirty four (71%) isolates were cultured from 48 rectal swabs from Kenya in 1996. Twenty six isolates were Shigella dysenteriae; eight were Shigella flexneri. The antibiotic sensitivity patterns of the two species were similar and are shown in Figure 8. The isolates were sensitive only to nalidixic acid and furazolidone.

DISCUSSION

The low yield (20%) of positive cultures obtained in 1994 was attributed to the long time intervals (often more than one week) between collection of specimens and receipt at the laboratory, and to poor storage during transport. In 1995, the AMREF laboratory produced guidelines on specimen collection, storage and transportation and provided transport media to all health agencies on request; since then the yield has increased to over 40%.

During the cholera outbreak in Kenya in 1971, surveillance was undertaken in all affected districts. The reported isolates were mainly serotype Inaba with clusters of Ogawa particularly close to the Uganda border(7). Thirteen thousands six hundred and seventy four people received tetracycline treatment during a mass chemoprophylactic campaign in the Marsabit/Moyale areas of northern Kenya(8).

Isolates investigated from cholera outbreaks in Kenya during the 1970’s and 1980’s were biotype El Tor and predominantly serotype Ogawa. All isolates during a cholera outbreak on the south Kenyan coast in 1981 were V. cholerae, biotype El Tor, serotype Ogawa(9). In a study in Homa Bay Hospital in western Kenya in 1982, the majority of isolates were resistant to tetracycline(10). In a further study in 1983 in Nyanza Province, the majority of strains were resistant to tetracycline and ampicillin, but all strains were sensitive to nalidixic acid and chloramphenicol(11). Our current findings in north eastern Kenya show that sensitivity to tetracycline has been retained, although there is 100% resistance to ampicillin.

All 102 isolates of V. cholerae studied during an outbreak on the east coast of Tanzania in 1977/1978 were biotype El Tor, serotype Ogawa(12). During the first month of the study, all isolates were sensitive to tetracycline and chloramphenicol, and 85% were sensitive to ampicillin. After five months of antimicrobial use for treatment of cholera and mass prophylaxis using tetracycline, resistance to tetracycline had risen to 76%, resistance to ampicillin was 86% and to chloramphenicol was 52%. Our findings from western Tanzania in 1994 and 1995 showed 100% resistance to these antibiotics.

A major outbreak of cholera occurred in 1984 amongst Rwandan refugees living in camps in eastern Zaire. There were an estimated 23,800 deaths among 47,500 suspected cases. Siddique et al isolated V. cholerae O1, biotype El Tor, serotype Ogawa, from 61% of 13 samples collected(5). The organisms were sensitive only to erythromycin, ciprofloxacin, furazolidone and mecillinam. These findings
are not consistent with our own findings of 100% sensitivity to nalidixic acid in Rwanda in 1994, although in both cases the findings are based on a small number of isolates.

Maimone et al. (13), found that V. cholerae (Ogawa) isolates in the north-west region of Somalia were sensitive to the drugs tested including tetracycline, while isolates from the other regions were resistant. In this study, V. cholerae isolates from Mogadishu, showed varying susceptibility to tetracycline during the study period. Over 80% of isolates in 1994 and in 1995 were sensitive to tetracycline, as opposed to only 44% of isolates in 1995. Isolates from other sites in southern Somalia (Kismayu, Baidoa, Lower Shebelle) and from northern Somalia (Bosasso, Hargeisa, Baidera) retained >90% sensitivity to tetracycline throughout the study period. These findings suggest that pockets of tetracycline resistant isolates exist in Somalia.

In severe cases of cholera, antibiotics reduce the volume and duration of diarrhoea. Wide and indiscriminate use of antibiotics leads to rapid development of resistance. Antibiotics are now only recommended in the treatment of severe disease, with selective chemoprophylaxis given to close contacts if the secondary attack rate is high (14).

Early laboratory analysis is essential to confirm the presence of disease, to determine the characteristics of the organism and to establish antibiotic sensitivity patterns, so that effective treatment and control measures can be instituted as rapidly as possible.

The majority of endemic cases of Shigella dysentery in developing countries are caused by Shigella flexneri, with Shigella dysenteriae type I being responsible for the most severe type of epidemic shigellosis. Shigella sp. rapidly acquire drug resistance, especially where antimicrobials are widely used for the treatment of diarrhoea.

The epidemic in the Lakes Region (Zaire, Rwanda, Burundi and western Uganda) which started in 1979 was caused by multiple drug resistant S. dysenteriae type 1 which also developed resistance against nalidixic acid during the epidemic (3). During the nationwide barriary dysentery epidemic which started in Tanzania in 1981, 207 patients in Dar es Salaam were investigated (4). Multiple drug resistant S. dysenteriae type 1 was the most commonly isolated organism. In the outbreak of dysentery in Zaire in 1984, S. dysenteriae type I was isolated, sensitive only to ciprofloxacin and mexitilam (5). Analysis of antibiotic resistance patterns of Shigella isolates in the region, including those in our own study, have shown increasing and widespread resistance to tetracycline, ampicillin and chloramphenicol (15-17).

Transmissible plasmid encoded antimicrobial resistance is the major route of dissemination of multiple antimicrobial drug resistance. Resistance may be spread among related species, and even between different genera of bacteria (17). Mhalu et al. (4) demonstrated plasmid conferring resistance in S. dysenteriae type I, and also showed that the multiple drug resistance of S. flexneri was due to plasmids which were transferable wholly or partially to K 12 E. coli. Our findings of a similar sensitivity pattern of both S. dysenteriae and S. flexneri suggest transfer of multiple drug resistance plasmids between the two species.

Appropriate antimicrobial therapy decreases the severity and duration of Shigella dysentery. Due to the increasing ineffectiveness of commonly available antibiotics, drugs such as the fluoroquinolones are now being utilized for treatment (18), although they are costly and their safety in children has not been established. It is vital that antibiotics should only be used in severe cases of Shigella dysentery and after establishment, wherever possible, of antibiotic sensitivity patterns.

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