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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *VIBRIO CHOLERAE* 01 STRAINS DURING TWO CHOLERA OUTBREAKS IN DAR ES SALAAM, TANZANIA

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ABSTRACT

Objective: **To determine and compare the antimicrobial susceptibility patterns of *Vibrio cholerae* 01 strains, which were isolated in two cholera epidemics in 1997 and 1999 in Dar es Salaam.**

***Methods: V. cholerae* 01 strains isolated from patients with cholera in Dar es Salaam city during 1997 (94 isolates) and 1999 (87 isolates) were stored on nutrient agar slants at room temperature and antimicrobial susceptibility pattern was determined, using Kirby Bauer method.**

Setting: **Department of Microbiology and Immunology, Muhimbili Medical Centre, Dar es Salaam, Tanzania.**

Results: **A total of 181 *V. cholerae* 01 strains were studied during two epidemic periods when tetracycline or erythromycin was used for treatment of patients with severe disease. Among the 94 *V. cholerae* Ol strains isolated in 1997; 98.6%, 93.6%, 83%, 81.9%, 36.2%, 35.5%, 3.2% were sensitive to ciprofloxacin, tetracycline, ampicillin, erythromycin, nalidixic acid, chloramphenicol and trimethoprim/ sulphamethoxazole, respectively. Among the 87 *V. cholerae* 01 isolates collected in 1999, 100%, 58.6%, 46.0%, 46%, 47.1%, 19.5%, 3.4% were sensitive to ciprofloxacin, tetracycline, ampicillin, erythromycin, chloramphenicol, nalidixic acid and trimethoprim/sulphamethoxazole, respectively. Between 1997 and 1999, there was a significant increase in the proportion of *V. cholerae* 01 isolates resistant to tetracycline, ampicillin, nalidixic acid and to erythromycin but there was no change for susceptibility to ciprofloxacin and trimethoprim/ sulphamethoxazole.**

Conclusion: **Significant proportion of *V. cholerae* 0l strains in Dar es Salaam were resistant to commonly used antimicrobial agents during the two years of the study. Therefore, there is a great need to control the utilisation of antimicrobial agents in cholera control, in addition to continuing carrying out surveillance of antimicrobial resistance as a guide to choice of antimicrobial treatment. Rotational use of the available drugs with regular monitoring of susceptibility may contribute to continuing usefulness of such drugs.**

INTRODUCTION

Vibrio cholerae (V. cholerae) 01 the causative organism of cholera was first described by Robert Koch in 1883(1). Of recent, *V.* cho*lerae* 0139 has also been associated with severe forms of cholera(2). Notification and surveillance systems of infectious diseases including that caused by *V.* cho*lerae* 01 in most developing countries is lacking, but available epidemiological and clinical evidence show that cholera is a public health problem(3). Throughout history, *V. cholerae* 0l has caused repeated epidemics in various areas of the world, especially in Asia, the Middle East and Africa(3). The first recorded major cholera epidemic in Tanzania during the twentieth century was reported in 1974, followed by the next epidemic that occurred in 1977, and since then, the disease has remained endemic with sporadic epidemics occurring mainly during the rainy seasons in areas with poor sanitation(4,5). Fluid and electrolyte replacement is the main stay of treatment of cholera patients, however, patients with severe disease require antibiotic treatment to reduce the duration of illness and reduce replacement fluid intake. Like most bacteria of clinical and public health significance, *V. cholerae* 01 is continuously becoming more resistant to a variety of antimicrobial agents, necessitating use of newer drugs which are more expensive and have more adverse effects to patients.

Spread of cholera epidemics worldwide has been associated with the emergence of multiple drug resistance among a large number of *V.* chole*rae* 01 strains(2,4,6). Literature on the antibiotic susceptibility of cholera organisms from most developing countries is patchy. Worldwide, *V. cholerae* 01 strains resistant to tetracycline, trimethoprim/sulphamethoxazole and ampicillin are common(6-10). In many of these studies, the main reasons for the rapid rise in antimicrobial resistance have been extensive antimicrobial prophylaxis, unauthorised dispensing and use of these agents in animal husbandry(11,12).

In Tanzania, data collected during cholera epidemics in 1990 and 1991 showed that all *V. cholerae* 01 strains were sensitive to erythromycin, kanamycin, gentamicin and ciprofloxacin(7). In a recent study on antimicrobial susceptibility of *V.* cholerae 01 isolates from eastern African countries between 1994 and 1996, about 80% to 90% of the isolates in Kenya and southern Sudan, and 65 to 90% of isolates in Somalia were sensitive to tetracycline(9). However, during the same period, 100% of isolates from Tanzania and Rwanda were resistant to tetracycline(9) and the percentage of trimethoprim/ sulphamethoxazole and chloramphenicol sensitive strains decreased from 85% to 10% during the same period(9). In a recent study in India, most *V. cholerae* 01 isolates were sensitive to tetracyline and gentamicin but were highly resistant to trimethoprim/sulphamethoxazole(13)

Antimicrobial resistance is therefore a global public health problem. The increase in the magnitude of bacterial species resistant to multiple antimicrobial agents have shown linear relationship with the amount of antimicrobial agents dispensed in a particular hospital or community for treatment or prophylaxis (8, 9). We are reporting on the comparison of the antimicrobial susceptibility patterns of *V. cholerae* 01 isolates that were prevalent in the cholera epidemics of 1997 and 1999 in Dar es Salaam city, Tanzania.

MATERIALS AND METHODS

Study samples: The study was conducted at the Department of Microbiology and Immunology, Muhimbili Medical Centre, which is the national referral hospital in Tanzania. The study samples included *V. cholerae* 01 strains obtained from stool samples submitted from patients treated in various hospitals in Dar es Salaam, between May and June 1997 and between January and May 1999 covering the main rainy season in the city. The strains were stored on plain nutrient agar slants at room temperature (22°C) with three monthly subculture and subsequently subcultured on nutrient agar for antibiotic susceptibility testing.

Laboratory methods: Stool samples were inoculated onto Thiosulphate-Citrate-Bile-Salt (TCBS) agar, incubated at 37°C for 24 hours. Suspected *V. cholerae* 01 isolates were identified based on characteristic colonial morphology, motility, and agglutination with specific *V.* c*holerae* polyvalent 01 and *V. cholerae* serotype Ogawa and Inaba antisera. Non-agglutinating characteristic colonial growth was subjected to biochemical tests and if positive were named *V. cholerae* non-01 and were not processed further. Outbreaks of *V. cholerae* 0139 have not yet been described in East Africa in spite of continuing surveillance for the organism.

Antimicrobial susceptibility testing was done using the Kirby-Bauer method(14). Briefly, five colonies of *V.* c*holerae* 01 were lightly touched with a loop and inoculated in a tube containing Muller Hinton broth and incubated for a few hours at 35°C until the suspension became slightly turbid and then diluted with sterile saline to match the 0.5 Mac Farland turbidity standard (1.5 x 105 colony forming units/ml). Using a sterile cotton swab, an entire surface of dried Muller Hinton agar plate (15x 150 mm Petri dish) with 4mm of agar depth was streaked uniformly with the swab dipped in the standard V. *cholerae* suspension after squeezing off extra fluid on the walls of the tube. The inoculated plate was allowed to dry for about five minutes and the appropriate antibiotic disks were then applied using sterile forceps and incubated at 37°C overnight. The commercial antibiotic disks used included: tetracycline (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), ampicillin (10 µg), nalidixic acid (30 µg), trimethoprim/sulphamethoxazole (25 µg) and ciprofloxacin (5 µg). After the incubation, the inhibition zone diameters were measured using a transparent plastic ruler and compared with those of a control organism (*Escherichia coli* NCTC 25922), which was tested whenever a batch of *V. cholerae* 01 strains was tested. The inhibition zones were interpreted according to zone size by using a table that relates zone diameter to the degree of microbial resistance(15). Isolates that were moderately sensitive were taken as fully sensitive during analysis.

Data analysis: Proportions of the sensitive *V. cholerae* 01 isolated in 1997 and 1999 were compared using the Chi-squared test, and a two-sided p value less than 0.05 was used to indicate statistically significant differences.

RESULTS

A total of 181 V. *cholerae* 01 strains were studied comprising of 94 isolates collected in 1997 and 87 isolates collected in 1999. All patients came from the three districts of Dar es Salaam: Kinondoni, Temeke, and Ilala. Tetracycline or erythromycin was being used for treatment of patients with severe disease during the study period but no prophylaxis with these drugs was recommended at the time.

Table 1 compares the antimicrobial susceptibility patterns of the *V. cholerae* 01 isolates obtained in 1997 and 1999. Among the 1997 isolates, 73/74 (98.6%), 88/94 (93.6%), 78/94 (83%), 77/94 (81.9%), 34/94 (36.2), 33/94 (35.5%) and 3/94 (3.2%) were sensitive to ciprofloxacin, tetracycline, ampicillin, erythromycin, chloramphenicol, nalidixic acid and trimethoprim/sulphamethoxazole respectively. Antimicrobial susceptibility patterns of the *V. cholerae* 01 isolates collected in 1999 showed that, 86/ 86 (100%), 51/87 (58.6%), 40/87 (46.0%), 40/87 (46%) and 41/87 (47.1%) were sensitive to ciprofloxacin, tetracycline, ampicillin, erythromycin and chloramphenicol, respectively. Nevertheless, only 17/87 (19.5%) strains were sensitive to nalidixic acid while 3/94 (3.4%) isolates were sensitive to trimethoprim/ sulphamethoxazole.

Table 1

Comparison of antimicrobial susceptibility patterns of V. cholerae 01 isolates collected in 1997 and 1999 to various antibiotics in Dar es Salaam

Antibiotic	1997 No	1997 No sensitive(%)	1999 No	1999 No sensitive(%)	p value
Ciprofloxacin	74	73 (98.6)	86	86 (100)	0.67
Tetracycline	94	88 (93.6)	87	51 (58.6)	<0.001
Ampicillin	94	78 (83.0)	87	40 (46.0)	<0.001
Erythromycin	94	77 (81.9)	87	40 (46.0)	<0.001
Chloramphenicol	94	33 (35.5)	87	41 (47.1)	0.100
Nalidixic acid	94	34 (36.2)	87	17 (19.5)	0.013
Trimethoprim/ sulphamethoxazole	94	3 (3.2)	87	3 (3.4)	0.753

When the antimicrobial susceptibility patterns of the *V. cholerae* 01 isolates obtained in 1997 were compared with those in 1999, there was a significant increase in the proportion of isolates resistant to tetracycline, ampicillin, nalidixic acid and erythromycin and a sustained very high resistance to trimethoprim/sulphamethoxazole. However, there was no significant increase in the number of isolates resistant to ciprofloxacin in the two years.

DISCUSSION

Globally, most bacterial pathogens are becoming more resistant to commonly used antimicrobial agents. Majority of cholera patients are usually treated by replacement of fluids and electrolytes, and only a small proportion with severe disease require antibiotic treatment.

In the present study, more than 93% of *V. cholerae 01* strains isolated in Dar es Salaam in 1997 were sensitive to tetracycline but two years later the number of tetracycline resistant isolates had significantly increased by thirty five per cent. Similar significant increase in resistant strains was observed against ampicillin, nalidixic acid and erythromycin during the same period. Treatment guidelines given by the Ministry of Health in Tanzania discourage giving prophylaxis for contacts of cholera patients, therefore the significant increase in the proportion of *V. cholerae* 01 resistant to tetracycline and erythromycin which were being used to treat cholera patients could be attributed to antibiotic pressure. Moreover, tetracycline and trimethoprim/sulphamethoxazole may be among the most commonly used drugs in the population for other common infections. However, majority of the *V. cholerae* 01 *s*trains during the study period in Dar es Salaam remained sensitive to ciprofloxacin which was recently introduced and is more expensive.

There have been several reports of *V. cholerae* 01 isolates resistant to multiple antimicrobial agents in the African region including Tanzania(6-10). Clinical observations have shown that when antibiotic pressure on a given epidemic is reduced, resistant bacterial strains tend to be replaced by sensitive ones (this observation is made by one of the authors, FSM). The current study has shown a significant increase in the percentage of *V. cholerae* 01 isolates resistant to antimicrobial agents, some of which are extensively used in Tanzania in agreement with other published reports(8-11). Worldwide, there is great variation in the distribution of antibiotic resistant *V. cholerae* 01 strains(3,11,12,17). Our results suggest that the antimicrobial susceptibility patterns of *V. cholerae* 01 cannot be predicted easily. In Tanzania, like in many other developing countries, there is no control in the prescription of antimicrobial agents and that antibiotics are also widely used in animal husbandry, which may account for the problem of increasing spread of antimicrobial resistance.

In conclusion, a significant proportion of *V. cholerae* 01 isolates in Dar es Salaam are becoming resistant to commonly used antimicrobial agents over time. Of utmost importance is the need to establish regular nationwide antibiotic susceptibility surveillance of *V. cholerae* 01 in different parts of the country in order to provide guidance on the best options in different situations. From this study, it is clear that rotational use of anti-cholera antibiotics may lead to emergence of fully susceptible strains over time, which may allow for extension of use of the most effective therapies such as tetracycline.

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