Plasmid characterization of drug-resistant *Shigella dysenteriae* 1 from an epidemic in Central Africa

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SUMMARY

A widespread epidemic of severe dysentery in Zaire and neighbouring Central African countries was caused by a multiply drug-resistant strain of *Shigella dysenteriae* 1. Early isolations were resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines (R-type = ACSSuT). Later in the epidemic strains resistant to trimethoprim (Tm) became prevalent and a few strains resistant to kanamycin (K) or nalidixic acid were also isolated. All resistances except nalidixic acid were encoded by plasmids of incompatibility groups X (ACT) or I₁ (ACSSuTTm) and the epidemic strain also carried an SSu plasmid and a number of cryptic plasmids. The Inc X plasmid from this epidemic is the same as that in *Sh. dysenteriae* 1 strains isolated in Somalia in 1976 whereas the epidemic strains from the Shiga outbreaks in Central America, 1969 to 1971, and Sri Lanka, 1979, carried plasmids of group B. This epidemic demonstrates that when a multiresistant strain includes resistance to trimethoprim, nalidixic acid is a suitable alternative therapeutic agent.

INTRODUCTION

Bacillary dysentery caused by *Shigella dysenteriae* type 1 (Shiga) can result in a severe illness in which antibiotic therapy may be essential. There have been a number of large outbreaks of Shiga dysentery in which the epidemic strain carried plasmid-mediated resistance to those antibiotics usually recommended for treatment. The most extensive of these outbreaks occurred in Central America between 1969 and 1971 (Mata *et al.* 1970) and was caused by a strain resistant to chloramphenicol, streptomycin, sulphonamides and tetracyclines (resistance pattern or R-type CSSuT). In an outbreak in Mexico City in 1972 the strain carried the same plasmid coding for CSSuT resistance, as well as a plasmid conferring resistance to ampicillin (A) (Olarde, Filroy & Galindo, 1976). Similarly, there have been outbreaks of severe Shiga dysentery in India in 1978 (Macaden *et al.* 1980),

Bangladesh, 1972-73 (Crosa *et al.* 1977) and Sri Lanka, 1976 (Velauthapillai, personal communication); in all of these the predominant R type was either CSSuT or ACSSuT.

Until recently there have been few reports of drug resistance in *Sh. dysenteriae* type 1 from Africa. An epidemic in Somalia in 1963-64 was caused by a strain resistant to streptomycin, sulphonamides and erythromycin and many non-epidemic strains isolated in 1976 were multiresistant (Mero, 1976).

Since the beginning of 1980 there has been a widespread outbreak of Shiga dysentery in Central Africa (Frost *et al.* 1981; Frost, Rowe & Vandepitte, 1982; Malengreau *et al.* 1983). This started in North East Zaire near the border with Uganda and has spread through Zaire, Rwanda and Burundi. Early isolations were resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines (R-type = ACSSuT). In July 1981 tetracycline was replaced by cotrimoxazole as the therapeutic agent of choice, and since August 1981 the majority of strains isolated in Zaire, Rwanda and Burundi have been resistant to trimethoprim (R-type = ACSSuSpTTm).

In November 1981 treatment with nalidixic acid was introduced and the outbreak has since declined in Zaire with the case fatality rate dropping to 1.1% in April-July 1982 from a peak of 4.6% in August-October 1981 (Malengreau *et al.* 1983).

In the latter part of 1982 we received one strain resistant to nalidixic acid (R-type ACSSuSpTTmNx) isolated in Zaire and several resistant to kanamycin (R-type ACKSSuTTm) isolated in Rwanda.

In all of these epidemic strains of *Sh. dysenteriae* type 1 the drug resistance has been plasmid-mediated, except for resistance to nalidixic acid. Both the Central American and the majority of South East Asian strains carry plasmids belonging to incompatibility group B, whereas those from Central Africa carry plasmids of groups X and I₁ (Frost *et al.* 1982). The plasmids present in strains from Central Africa and Somalia have been compared with each other and with strains from other parts of the world in an attempt to relate the plasmid content of the strains to their geographical origins.

MATERIALS AND METHODS

Strains examined

Sh. dysenteriae type 1 isolated in Zaire and Rwanda in 1981 and 1982 (Frost *et al.* 1981, 1982) were compared with strains isolated elsewhere. Strain numbers, place of isolation and R-type are given in Table 1.

Drug resistance, resistance transfer and plasmid characterization

All strains were R-typed and tested for resistance transfer using the methods of Anderson & Threlfall (1974). Resistance plasmids were transferred to a standard strain of *Escherichia coli* K12 (DEP ref. 14R525) and tested for incompatibility with standard plasmids of the following incompatibility groups (Inc): B, C, F_I, F_{II}, F_{IV}, H, I₁, I₂, J, K, M, N, P, W, X (Jacob *et al.* 1977) and F_Ime (Anderson *et al.* 1977). Non-autotransferring SSu plasmids were tested for compatibility with the major group of SSu plasmids which includes NTP2 (Anderson *et al.* 1968; Smith,

Table 1. *Plasmid content of Sh. dysenteriae 1 strains and E. coli derivatives*

<i>Sh. dysenteriae</i> 1 strain	R-type*	Molecular weight of plasmid bands on agarose gel (× 10 ⁻⁶)							<i>E. coli</i> K12 derived by	R-type	Plasmid type	Molecular weight	
E7926 Somalia, 1976	ACSSuT	120	70	40	6.6	6.1	2.0	1.6	Conjugation Transformation	ACT SSu	IncX SSu	39 5.8†	
E22106 Zaire, 1981	ACSSuT	120	42	6.9	5.3	4.1	2.1	1.7	Conjugation	ACT	IncX	38	
E23567 Zaire, 1981	ACSSpSuTTm	115	80	41	6.9	5.4	4.2	2.2	1.7	Conjugation Transformation	ACSSpSuTTm ACT SSu	IncI ₁ IncX SSu	78 39 4.2‡
E24198 Zaire, 1981	ACSSpSuTTm	120	76	40	6.7	5.3	4.3	2.4	2.2	Conjugation Transformation	ACSSpSuTTm ACT SSu	IncI ₁ IncX SSu	75 40 4.2‡
E24791 Zaire, 1981	ACT	120	38	6.1	4.7	2.0	1.8		NT§	—	—	—	
E26214 Rwanda, 1981	ACSSpSuTTm	110	76	39	6.2	4.0	3.2	2.1	1.7	NT	—	—	
E26527 Zaire, 1982	ACSSpSuTTmNx	110	83	45	4.2	9.6	5.4	4.2	2.2	1.8	ACT	IncX	45/42
E27940 Rwanda, 1982	ACKSSuTTm	110	60	42	8.6	6.6	5.4	4.5	2.3	< 1.5	ACT AKSSuT ACSSuTTm ACKSSuTTm	IncX IncX IncI ₁ IncI ₁	40 45 60 60
Strains from non-African sources													
40R289 C. America, 1969	CSSuT	120	78	6.8							CSSuT	IncB	78
E1012 Mexico, 1974	CSSuT	115	83	6.5							CSSuT	IncB	78
E16171 Sri Lanka, 1979	CSSuT	120	59	6.6	4.1	2.1	1.7				CT SSu	IncB SSu	56 4.1†
* Symbols for drug resistance: A, ampicillin; C, chloramphenicol, K, kanamycin; S, streptomycin; Sp, spectinomycin; Su, sulphonamides; T, tetracyclines; Tm, trimethoprim; Nx, nalidixic													

* Symbols for drug resistance: A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Sp, spectinomycin; T, tetracyclines; Tm, trimethoprim; Nx, nalidixic acid.

† Plasmid encoding streptomycin and sulphonamides resistance belonging to the same incompatibility group as NTP2.

‡ Plasmid not belonging to the same incompatibility group as NTP2.

§ *E. coli* K12 progeny not tested.

Humphreys & Anderson, 1974), R300B (Barth & Grinter, 1974) and RSF1010 (Grinter & Barth, 1976); using a laboratory-derived ASu plasmid, NTP3. The methods used were based on those of Grindley, Grindley & Anderson (1972) and Anderson & Threlfall (1974).

Preparation of plasmid DNA, restriction endonuclease treatment and agarose gel electrophoresis

Plasmid DNA was prepared by the method of Birnboim & Doly (1979) from wild-type *Sh. dysenteriae* 1 strains and from *E. coli* K12 transconjugants and transformants. Shigella strains were killed with 1% diethylpyrocarbonate before DNA extraction. The partially purified plasmid DNA was dissolved in TE buffer (0.01 M Tris-hydrochloride pH 8.0 containing 0.001 M-EDTA) and examined directly by electrophoresis on vertical slab gels containing 0.6% (w/v) agarose as described previously (Willshaw, Smith & Anderson, 1979). The molecular weights of the plasmids present were determined by reference to plasmids of molecular weight 2.6×10^6 to 78×10^6 run on the same gels.

Where appropriate, plasmid DNA was digested with *Eco*R1 restriction endonuclease for 4–5 h at 37 °C under the conditions specified by the enzyme manufacturer (Bethesda Research Laboratories). Restriction fragments were separated on vertical 1% (w/v) agarose gels (Willshaw *et al.* 1980) and their molecular weights determined relative to fragments of phage DNA generated by treatment with *Eco*R1 plus *Hind* III. Fragments smaller than about 0.5×10^6 were not measured.

Plasmid transformation

Plasmid DNA prepared from *Sh. dysenteriae* 1 strains as described by Birnboim & Doly (1979) was used for transformation of *E. coli* K12 by the method of Dagert & Ehrlich (1979). Transformant lines carrying drug-resistance plasmids were selected on nutrient agar containing appropriate antibiotics.

RESULTS

Table 1 shows the number and sizes of the plasmids carried by drug-resistant *Sh. dysenteriae* 1 strains from different sources. Drug resistance plasmids were identified in representative strains by direct transfer (or mobilization) of resistance markers into *E. coli* K12 (Frost *et al.* 1981) or by transformation of *E. coli* K12 to drug resistance using plasmid DNA prepared from the wild type *Sh. dysenteriae* 1 strains.

All the strains carried at least three plasmids and those isolated from the African countries showed between 6 and 10 plasmid DNA bands detected by agarose gel electrophoresis. It was not possible with certainty to conclude that all the bands represented independent plasmids since, particularly for plasmids of molecular weight less than 10×10^6 , open circular, and linear molecules may be detectable in addition to the covalently closed circular DNA molecules (Willshaw *et al.* 1979). For the African strains the results recorded in Table 1 represent the plasmid profiles that were reproducibly observed. In some cases the absence of a plasmid in a wild type strain or its introduction into K12 enabled linear and open circular DNA forms to be identified.

The results show that only one plasmid, of molecular weight $110\text{--}120 \times 10^6$, appeared to be common to all the strains examined and was observed even in isolates that had been maintained in the laboratory for more than 10 years.

Correlation of plasmid profile with geographical source

(1) *Strains from Somalia and Central Africa*

The Somalian strain E7926 was one of a group isolated at least 5 years before the cultures associated with the epidemic of Shiga dysentery in Zaire and its neighbouring countries (Frost *et al.* 1981). However, all these African strains showed significant similarities in their plasmid content (Table 1) and can be considered in relation to each other.

(a) *Plasmids of unknown function.* All the strains from these sources carried at least three cryptic plasmids of molecular weight less than 10×10^6 . Strain E7926 represents the simplest example in which molecular species of 6.6, 2.0 and 1.6 were observed (Table 1). Plasmids of approximately these sizes were also present in the strains from Zaire and other African countries, the only exception being E26527 in which the species of 6×10^6 was absent. This common pattern of cryptic plasmids was augmented in the strains from Central Africa by the presence of a plasmid of molecular weight 5.3×10^6 in all isolates except E24791 from Zaire and E26214 from Rwanda.

Strains isolated later in the course of the epidemic appeared to have acquired one or two additional cryptic plasmids. Thus E26527 and E27940 carried a plasmid of molecular weight $8.6\text{--}9.6 \times 10^6$ and the latter also possessed a very small plasmid of molecular weight less than 1.5×10^6 . Despite the increasingly complex plasmid profile of the strains, the pattern of carriage of the small cryptic plasmids was similar in strains isolated at different times and from different countries.

(b) *Drug resistance.* All the strains examined were resistant to between three and eight antimicrobial agents (Table 1).

Plasmids of Inc X encoding ACT

Genetic studies had indicated that the majority of the drug resistant *Sh. dysenteriae* 1 strains isolated from Somalia in 1976 and the strains from Zaire carried a plasmid belonging to incompatibility group X and encoding ACT (Frost *et al.* 1981). In E7926 this plasmid was identified as being of molecular weight 40×10^6 by transfer of the plasmid into *E. coli* K12. A plasmid of approximately this size was also present in all the other *Sh. dysenteriae* 1 strains from Zaire and neighbouring countries and in several cases this was confirmed as coding for ACT and belonging to group X (Frost *et al.* 1981). In one strain (E26527) plasmids of 45 and 42×10^6 were observed. A K12 transformant of R-type ACT carrying a plasmid of group X also showed two plasmid DNA species. We concluded that in E26527 the group X plasmid may be slightly larger and give rise spontaneously to a smaller DNA species.

DNA preparations of the Inc X plasmids from strains E7926, E22106, E23567, E24198 and E26527 were digested with the restriction enzyme *Eco*R1. All the plasmids gave indistinguishable digest patterns with fragments of molecular weight ($\times 10^6$) of > 15 , 5.6, 5.1, 3.7, 1.89, 1.53, 1.31, 1.18 and 0.80. This result showed that the Inc X plasmids in the Zaire strains were identical, and that they were indistinguishable from the X group plasmid from the earlier Somalian culture.

Plasmids encoding SSu

In a previous study, it was reported that strains such as E7926 from Somalia carried a non-conjugative resistance plasmid encoding SSu (Frost *et al.* 1981). This plasmid appeared to be mobilized into K12 by the X-group plasmid from this strain. Total plasmid DNA from E7926 was used to transform *E. coli* K12 and streptomycin-resistant transformants were selected. These were also Su-resistant. Transformant lines (Table 1) carried a single plasmid of approximately 5.8×10^6 which could be correlated with the presence of a plasmid band in the wild type strain. This plasmid was incompatible with the ASu resistance plasmid NTP3 described by Anderson *et al.* (1968).

In strains from Zaire, it was reported previously that resistance to streptomycin and sulphonamides could not be transferred or mobilized into *E. coli* K12. Total plasmid DNA preparations from representative strains E23567, E24198 were therefore used for transformation of K12 as described above. Transformant colonies from these experiments carried a single plasmid of 4.2×10^6 which could be identified in the wild-type strain (Table 1). This plasmid was compatible with NTP3. No SSu transformants were obtained that carried a larger plasmid as in E7926, but all the other Central African strains also carried similar plasmids of $4.0\text{--}4.5 \times 10^6$.

Plasmids of Inc I₁ encoding ACSSuSpTTm

During the course of the epidemic of Shiga dysentery in Zaire trimethoprim resistant isolates were observed; probably emerging in response to selective pressure due to the use of this drug in treatment (Frost *et al.* 1982). Acquisition of Tm was correlated with the gain of a plasmid belonging to Inc I₁ and encoding ACSSuSpTTm. E23567, E24198 and E26527 (see below) are examples of strains of this type from Zaire; E26214, of the same R-type was isolated after the spread of the epidemic to Rwanda.

In these strains, the plasmid carrying trimethoprim resistance had a molecular weight of approximately 80×10^6 (see E23567, Table 1). DNA of the ACSSuSpTTm plasmids, from E23567 and E24198 from Zaire and E26214 from Rwanda, were treated with the restriction enzyme *Eco*R1. The digest patterns of the plasmids were indistinguishable and the sizes of the fragments were ($\times 10^{-6}$): > 15, 10.8, 8.1, 6.1, 5.2, 4.8, 4.2, 4.1, 2.79 ($\times 2$), 2.20, 2.02 ($\times 2$), 1.88, 1.39, 1.33, 1.27, 1.12, 0.97, 0.80, 0.69. Thus these strains carried identical Inc I₁ plasmids.

Resistance to nalidixic acid

In Zaire, one strain, E26527 was resistant to nalidixic acid and its plasmid profile is shown in Table 1. The drug resistance plasmids carried by this strain were Inc X (ACT), Inc I₁ (ACSSuSpTTm) and an SSu plasmid as described above. Attempts were made to use plasmid DNA from this strain to transform *E. coli* K12 to nalidixic acid resistance. Conjugation experiments were also performed to try to transfer or mobilize this marker into K12. Neither approach yielded Nx-resistant colonies and we concluded that this marker was probably chromosomal.

Resistance to kanamycin

In December 1983 a few kanamycin-resistant isolates were received from Rwanda (R-type = ACKSSuTTm); E27940 was examined in detail. *E. coli* K12

transconjugants resistant to ACT or AKSSuT carried a group X plasmid, while those of R-types ACKSSuTTm or ACSSuTTm carried group I₁ plasmids (Table 1). The group I₁ plasmids from E27940 were smaller than those found previously (Table 1) and did not code for resistance to spectinomycin. Restriction enzyme digest of these plasmids gave fragment sizes as follows ($\times 10^6$); R-type ACKSSuTTm: $> 15(\times 2)$, 9.6, 5.3, 5.1, 4.9, 4.4, 3.3, 1.93, 0.95 and 0.82; R-type ACSSuTTm: $> 15(\times 2)$, 9.0, 5.7, 4.4, 3.3, 2.4, 1.93, 0.95 and 0.82. Thus these two plasmids have at least 8 out of 11 fragments of similar size and are probably variants of the same plasmid. This plasmid has few fragments in common with those Inc I₁ plasmids encoding the R-type ACSSuSpTTm typified by the plasmid from E23567.

Since kanamycin resistance could be recovered on both the X and I₁ plasmids in tranconjugants, its location in the wild-type strain is uncertain.

(2) Strains from other sources

(a) *Central America and Mexico*. The two strains examined were of R-type CSSuT and in both the resistance markers were carried on a single plasmid of molecular weight 75–80 $\times 10^6$ (Table 1). This plasmid has been shown to belong to Inc B (Grindley *et al.* 1972; Frost *et al.* 1981). Like all the other *Sh. dysenteriae* 1 strains in this study the strains carried a very large plasmid of approximately 120 $\times 10^6$ (see above). A cryptic plasmid of 6.5–6.8 $\times 10^6$ was also present. A species of similar size was carried by all but one of the African strains but we do not know if the plasmids are related. The strains from this source were clearly distinguishable by the absence of other small cryptic plasmids or SSu plasmids.

(b) *Sri Lanka*. Strain E16171 (Table 1) also carried a plasmid of Inc B that coded for CT (Frost *et al.* 1981). Examination of an *E. coli* K12 strain carrying this plasmid showed that it had a molecular weight of 59 $\times 10^6$. It had been shown previously that E16171 carried an independent SSu plasmid that could be co-transferred with the Inc B plasmid (Frost *et al.* 1981). This SSu plasmid was introduced by itself into K12 by transformation and was shown to have a molecular weight of 4.1 $\times 10^6$ (Table 1); in this respect it resembled SSu plasmids found in *Sh. dysenteriae* 1 strains from Zaire and other Central African countries but differed from the SSu plasmid in E7962 from Somalia.

Strain E16171 carried a cryptic plasmid of molecular weight 6.6 $\times 10^6$, similar in size to that found in all but one of the strains in Table 1 (see above). However, E16171 differed from the Central American and Mexican strains in possessing two additional cryptic plasmids of sizes that were characteristic of the Somalian and Central African strains (molecular weight 2.1 and 1.7 $\times 10^6$ (see Table 1). Thus in possessing a drug resistance plasmid of Inc B, E16171 was differentiated from the strains from Africa in which this plasmid type was not encountered; however, on the basis of its SSu and cryptic plasmids detected by gel electrophoresis it had distinct similarities to the African strains.

DISCUSSION

The epidemic of Shiga dysentery in Central Africa has spread throughout a wide area in a period of two to three years. Genetic studies on the plasmids carried by the causative organisms and analysis of the overall plasmid content of the

isolations received enabled changes to be followed during the course of the epidemic. There are very close similarities in the patterns of carriage of drug resistance plasmids and cryptic plasmids in all the *Sh. dysenteriae* 1 strains from Central Africa. It is likely that this reflects the spread of a single clone in this region and that plasmid content changed in response to antibiotic selection pressure. Generally there appeared to be an increasingly complex plasmid profile associated with later isolates although the conjugative Inc X plasmid and non-conjugative SSu plasmid ($ca. 4 \times 10^6$) were constant and characteristic markers. Non-conjugative SSu plasmids of a similar size have been isolated from *E. coli* in Europe and Egypt (van Treeck *et al.* 1981). This plasmid is compatible with NTP2.

The plasmid profiles of strains isolated in Somalia some years earlier than those from Central Africa had several similarities to those of the Zaire strains and the Inc X plasmids carried by the strains were indistinguishable by restriction enzyme digestion. However, the SSu plasmid in the Somalian strains was larger ($ca. 6 \times 10^6$) than that in strains from Zaire and was incompatible with NTP2 and thus belongs to this group of non-conjugative SSu plasmids (Anderson & Lewis, 1965; Smith, Humphreys & Anderson, 1974) which is widely distributed in salmonellae.

Like the Inc X plasmids, the group I₁ plasmids coding for resistance to ACSSuSpTTm isolated in both Zaire and Rwanda were indistinguishable by restriction enzyme digests. However, the plasmid from the kanamycin-resistant strain, E27940, was smaller and could be differentiated from the more typical I₁ plasmid by restriction enzyme digest. Kanamycin resistance was only found in a small number of strains isolated in Rwanda and these had apparently acquired a different Inc I₁ plasmid from that more commonly observed.

Examination of plasmid profiles of strains from other geographical areas illustrated that it was possible to distinguish clearly between strains from Central America and Central Africa. Although the strains from Sri Lanka, Somalia and Central Africa appeared to have four or five plasmids of similar size, genetic studies showed that the Sri Lankan strains resembled those from Central America in possessing an Inc B plasmid rather than the Inc X plasmid characterizing strains from Africa. However, the SSu plasmid in the Sri Lankan strain was the same size ($ca. 4 \times 10^6$) as that in Central Africa.

All *Sh. dysenteriae* 1 strains examined in this study carried a plasmid of molecular weight $110-120 \times 10^6$. It has been shown that plasmids of similar size have a role in the pathogenicity of shigella. Virulent strains of *Sh. flexneri*, *Sh. sonnei*, *Sh. boydii* and *Sh. dysenteriae* carry a plasmid of molecular weight $120-140 \times 10^6$ that encodes or regulates some of the functions required for invasion of epithelial cells (Kopecko, Washington & Formal, 1980; Kopecko *et al.* 1981; Silva, Toledo & Trabulsi, 1982). Watanabe & Timmis (1984) have shown that a 6×10^6 plasmid is also implicated in the pathogenicity of *Sh. dysenteriae* 1. A plasmid of MW 6 to 7×10^6 was demonstrated in all of the *Sh. dysenteriae* 1 strains in our study except E26527, a strain from Zaire that had acquired nalidixic acid resistance.

During the epidemic of Shiga dysentery in Zaire and its neighbouring countries an important feature has been the rapid adaptation of the causative organism to changes in antimicrobial therapy. The use of co-trimoxazole resulted in the spread of a strain carrying plasmid-mediated trimethoprim resistance. The occurrence of trimethoprim-resistant *Sh. dysenteriae* 1 has also been reported from Bangladesh

(Zaman *et al.* 1983), the United Arab Emirates (McCormack, 1983), Kashmir (Panhotra & Desai, 1983) and West Bengal (Pal, 1984). Preliminary studies on trimethoprim-resistant strains isolated in Britain from patients recently returned from India and strains received from the 1984 W. Bengal outbreak suggest that although trimethoprim resistance is transferable, resistance to other drugs is neither transferable nor mobilizable. In this respect they resemble multi-resistant strains of *Sh. flexneri* from India in which resistance to ACSSuT did not appear to be plasmid-determined (Frost & Rowe, 1983).

It is clear that the choice of a suitable antibiotic for Shiga dysentery is becoming severely limited. Ampicillin, chloramphenicol and tetracyclines are unlikely to be effective in Africa and Asia and resistance to trimethoprim is increasingly encountered. Nalidixic acid therapy has been used successfully in Central Africa (Malengreau *et al.* 1983) and in Kashmir (Panhotra & Desai, 1983). Although in Zaire some nalidixic acid resistance has occurred, it has not become widespread and there is no evidence that the resistance is plasmid-mediated. Until resistance became widespread ampicillin was an effective treatment for shigellosis; thereafter cotrimoxazole was recommended (Garrod, Lambert & O'Grady, 1981). When trimethoprim resistance occurs, nalidixic acid is an alternative therapy.

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