Association Between Azithromycin Therapy and Duration of Bacterial Shedding Among Patients With Shiga Toxin–Producing Enteroaggregative *Escherichia coli* O104:H4

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Context An outbreak of Shiga toxin–producing enteroaggregative *Escherichia coli* (STEC O104:H4) infection with a high incidence of hemolytic uremic syndrome (HUS) occurred in Germany in May 2011. Antibiotic treatment of STEC infection is discouraged because it might increase the risk of HUS development. However, antibiotic therapy is widely used to treat enteroaggregative *E coli* infection. In the German outbreak, a substantial number of patients received prophylactic azithromycin treatment as part of a therapeutic regimen with the C5 antibody eculizumab.

Objective To analyze the duration of bacterial shedding in patients with STEC infection who did and did not receive oral azithromycin therapy.

Design, Setting, and Patients At a single center in Lübeck, Germany, 65 patients with STEC infection, including patients with HUS as well as STEC-infected outpatients without manifestation of HUS, were investigated between May 15 and July 26, 2011, and were monitored for a mean of 39.3 days after onset of clinical symptoms.

Main Outcome Measure Carriage of STEC after azithromycin therapy.

Results Twenty-two patients received oral azithromycin and 43 patients did not receive antibiotic treatment. Among antibiotic-treated patients, long-term STEC carriage (>28 days) was observed in 1 of 22 patients (4.5%; 95% CI, 0%-13.3%) compared with 35 of 43 patients (81.4%; 95% CI, 69.8%-93.0%) who were not treated with antibiotics ($P<.001$). All 22 patients receiving azithromycin treatment had at least 3 STEC-negative stool specimens after the completion of treatment, and no recurrence of STEC was observed in these patients. As proof of principle, 15 patients who initially were not treated with antibiotics and were long-term STEC carriers were treated with oral azithromycin given for 3 days and subsequently had negative stool specimens.

Conclusion Treatment with azithromycin was associated with a lower frequency of long-term STEC O104:H4 carriage.

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Since May 2011, a large outbreak of Shiga toxin–producing *Escherichia coli* (STEC) has caused 3816 documented infections in Germany, including 845 confirmed cases of hemolytic uremic syndrome (HUS).[1] The outbreak strain was characterized as an extended-spectrum β-lactamase–expressing *E coli* of serotype O104:H4 with virulence factors of both enterohemorrhagic *E coli* (EHEC) and enteroaggregative *E coli* (EAggEC).[2-4]

In the recent outbreak, HUS developed in a large percentage of patients (22%), and 88% of the patients in whom HUS developed were adults.[1] According to existing recommendations, antibiotic treatment of STEC infection is discouraged because this therapy might increase the risk of HUS development.[5] However, antibiotic therapy is a widely accepted option for the treatment of traveler's diarrhea caused by EAggEC, and azithromycin is one of the antibiotics of choice.[6,7]

Patients colonized with enteropathogenic bacteria are considered infective

See also Patient Page.

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through fecal-oral transmission as long as pathogens can be detected in the stool. Because the outbreak strain expresses an aggregative adherence phenotype, bacterial shedding may be prolonged compared with classic EHEC.[8] Clinically, long-term carriage can cause persistent diarrheal symptoms.[9,10] Moreover, long-term carriers of enteropathogenic bacteria represent a chronic risk of human-to-human transmission and, therefore, their individual social and working life is legally restricted by the German health authorities, posing a high psychological and socioeconomic burden.

For the current outbreak, data on long-term STEC carriage have not as yet been published. In this study, we analyzed the duration of bacterial shedding in 65 patients, comparing those who received azithromycin with those without antibiotic treatment.

METHODS

Inclusion Criteria and Documentation

At the University Hospital of Lübeck, antibiotic therapy was withheld from patients with STEC unless clinically indicated (eg, severe sepsis). Patients with STEC-HUS received supportive therapy (hemodialysis and plasmapheresis) following the ad hoc guidelines of the German Society of Nephrology for treatment of typical HUS in adults.

However, based on recent clinical experience in 3 children with severe STEC-HUS,[11] eculizumab was administered to some patients. Eculizumab therapy was established according to the criteria of severe neurological involvement or persistence of HUS despite plasmapheresis therapy. Eculizumab interrupts the activation of the terminal complement complex; consequently, patients have an enhanced susceptibility to infection with meningococci.[12] Therefore, preventive treatment with azithromycin was recommended for 14 days (500 mg on days 1-3 and 250 mg on days 4, 6, 8, 10, 12, and 14) in addition to meningococcal vaccination.

Enrollment and inclusion of patients for the evaluation of the effect of azithromycin on STEC O104:H4 shedding was performed on June 24, 2011. Major inclusion criteria were detection of STEC O104:H4 (confirmed by polymerase chain reaction[1]) in clinically symptomatic or asymptomatic patients receiving or not receiving oral azithromycin with lack of other oral or systemic antibiotic therapy covering *E coli*. All patients were included if at least 2 microbiological results of stool specimens were available during a period of more than 28 days after onset of clinical symptoms (diarrhea or symptoms associated with HUS if diarrhea was lacking). In addition, patients with STEC infection who transitioned to confirmed noncarrier status were included independent of the duration of microbiological observations.

Confirmed noncarrier status was defined as 2 or more independent STEC-negative stool samples over a period of at least 6 days with no subsequent positive results. For treated patients, at least 1 negative stool sample had to be obtained after the end of treatment. All results of culture diagnosis of STEC O104:H4 were documented with regard to the onset of clinical symptoms, defined as day 1. Since the possibility of bacterial clearance and reinfection was assumed to be unlikely, any period before the last stool specimen tested positive for STEC was considered confirmed positive. Long-term carriage was defined as lack of negative stool samples on day 28.

For the observational part of the study (initial azithromycin therapy), patients gave written informed consent and the study was approved by the institutional review board of the University of Lübeck. Moreover, all patients with EHEC who had follow-up contact with our clinic provided written informed consent allowing EHEC-related history and clinical and biochemical data to be used for scientific purposes. For eradication therapy in long-term carriers, we obtained oral consent from each individual, documented by the attending physician. The prospective part of the study was performed in accordance with the local institutional review board's guidelines.

The 22 patients initially treated with azithromycin are part of a larger multicenter investigation evaluating eculizumab treatment for STEC-HUS, which was approved by all participating centers. All adult patients with HUS in the study reported herein who were not treated with azithromycin because of eculizumab for meningitis prophylaxis are included in a multicenter descriptive study examining *E coli* O104:H4–induced HUS. However, to date, reports from these studies have not been published.

Decolonization of Long-term Carriers

After post hoc confirmation of successful decolonization of patients who had received azithromycin for prophylaxis of meningitis, 15 adult patients with long-term carriage (>28 days) of STEC O104:H4 were treated as a proof of principle for 3 days with oral azithromycin (500 mg/d) for decolonization. These patients had not been treated with eculizumab as the primary indication for azithromycin prophylaxis and were asymptomatic for at least 3 weeks after their diarrheal episode. Serum lactate dehydrogenase and creatinine levels were determined prior to decolonization therapy and 4 to 7 days after end of treatment. Stool specimens were collected the day prior to prescription of antibiotic treatment and at least 3 stool specimens were investigated after the end of therapy.

Statistical Analysis

Mean values with standard deviations were calculated for age and time variables. Differences between subgroups were analyzed with the Mann-Whitney test. χ^2 Statistics were used for categorical data. The *t* test was used for comparison of laboratory data.

Survival function (in terms of the proportion of STEC carriers), probabilities, and 95% confidence intervals were estimated with Kaplan-Meier methods, using the onset of symptoms as the starting point. The confirmed noncarrier status was set as the outcome event, with the day of confirmation as the event date. This is the most conservative approach because it assumes that until the day of confirmation of noncarriage, a patient is deemed to be positive. However, it is likely that the patient already may have been a noncarrier several days before confirma-

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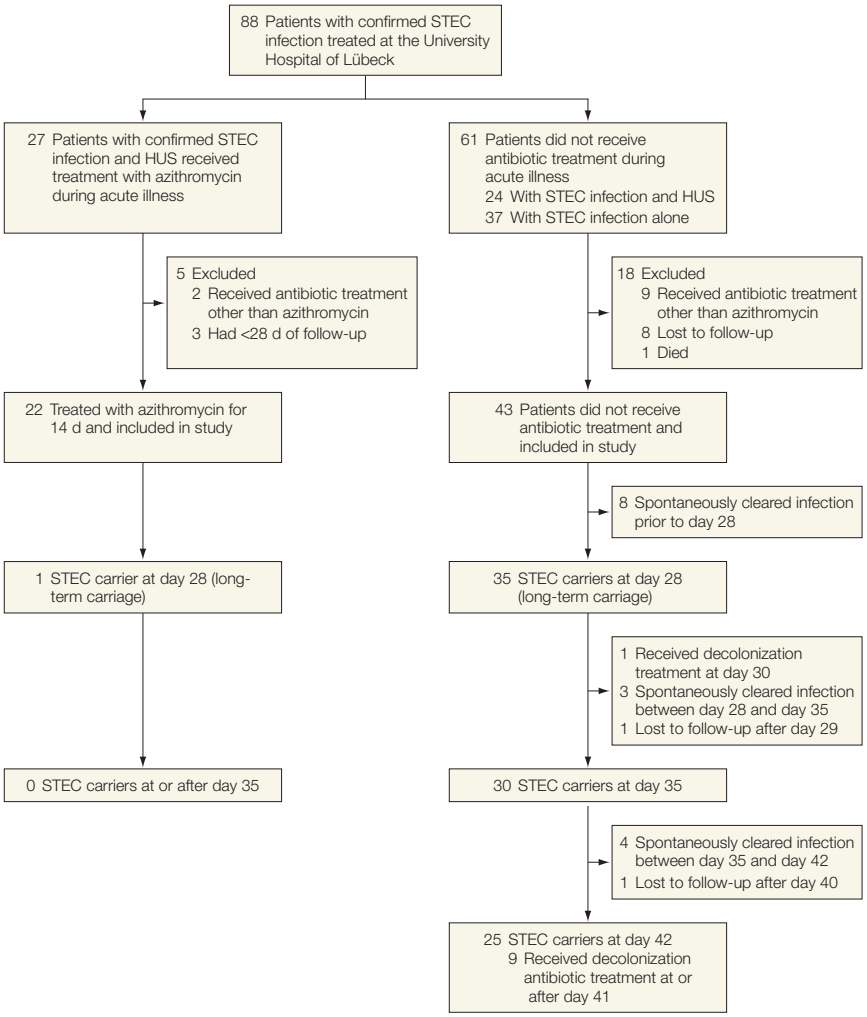
tion. To restrict the period to 42 days after onset of clinical symptoms, carriers at this day were censored. Groups with and without initial azithromycin therapy were compared using the log-rank test and a Cox regression model was fit to assess hazard ratio.

Level of significance was defined as 2-sided $P < .05$ for all analyses. Because of the acute outbreak situation, a power estimation and sample size calculation were not performed before patient enrollment. For statistical analyses, we used SPSS software, version 17 (SPSS Inc) and R software, version 2.14.0 (R Foundation for Statistical Computing).

RESULTS

As of June 24, 2011, all patients with confirmed STEC O104:H4 infection at the University Hospital of Schleswig-Holstein, Campus Lübeck, were recruited (n = 88). Twenty-three patients were excluded because they did not fulfill the inclusion criteria (FIGURE 1). Sixty-five patients met the inclusion criteria, with a mean observation period of 43.5 (SD, 10.5) days (TABLE 1). All results of culture diagnosis of extended-spectrum β-lactamase–producing STEC were documented with regard to onset of clinical symptoms (FIGURE 2).

Figure 1. Enrollment of Patients With STEC O104:H4 Infection Into the Study



STEC indicates Shiga toxin–producing enteroaggregative *Escherichia coli*.

STEC infections and HUS were predominantly observed in female patients (67.6%). The majority of patients were older than 18 years (mean age, 46.6 [SD, 19.7] years). Four of 5 children treated for STEC infection in our medical center could be included in the study. Thirty-seven patients fulfilled the clinical criteria of HUS according to the case definition of the Robert Koch Institute, including thrombocytopenia, hemolytic anemia, and acute renal dysfunction.[13]

The initial azithromycin-treated group included 22 HUS patients who had azithromycin therapy for meningitis prophylaxis during the first 2 weeks of therapy with eculizumab as recommended by the German Society of Nephrology. On average, patients treated with azithromycin started therapy 11.8 days after the onset of clinical symptoms (Table 1). The control group included 43 patients without antibiotic treatment, including 15 patients with HUS who improved clinically without eculizumab therapy and therefore did not receive azithromycin therapy, and 28 patients with extended-spectrum β-lactamase–producing STEC who did not have clinical signs of HUS. There were no significant differences in the age or sex distributions between the groups (Table 1).

Because of the lack of preexisting recommendations for microbiological diagnosis during acute illness, frequency of stool specimen analyses was not standardized from the beginning of the current STEC outbreak. On average, 6.6 (SD, 1.9) and 6.8 (SD, 3.2) samples (range, 2-17) were investigated per patient in the azithromycin group and control group, respectively, during the observed periods (Figure 2). The treatment and control groups were followed up for 40.7 (SD, 6.6) and 44.9 (SD, 11.8) days, respectively (Table 1).

The number of STEC carriers was significantly lower among patients treated with azithromycin (hazard ratio with azithromycin, 0.095; 95% CI, 0.041-0.218; $P < .001$; reference: no antibiotic treatment) (FIGURE 3). At day 21, rates of STEC carriage were 31.8% (95% CI, 12.2%-51.4%) in the initially treated group and 83.7% (95% CI, 72.7%-

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94.7%) in those not treated ($P<.001$). Long-term carriage (at day 28) was 4.5% (95% CI, 0%-13.3%) in the treated group and 81.4% (95% CI, 69.8%-93.0%) in the untreated group ($P<.001$). At day 35, no patient in the treated group was a STEC carrier and all patients remained STEC-negative after the completion of 14 days of treatment (Figure 2). In contrast, 25 of 43 patients (57.7%; 95% CI, 40.5%-75.0%) in the control group were STEC carriers at day 42 after onset of clinical symptoms. Individual patients not treated with azithromycin were STEC-positive for more than 50 days.

We compared patients who developed HUS with patients who had mild signs of infection (non-HUS) within the control group (TABLE 2). The HUS and non-HUS groups were observed for similar periods (HUS, 43.7 [SD, 8.4] days; non-HUS, 45.6 [SD, 13.4] days; $P=.83$). No significant differences in age or sex distributions were observed. The mean time of confirmed carriage was similar between the 2 groups, at 32.4 and 35.3 days in the HUS and non-HUS groups, respectively ($P=.98$) (Table 2).

The observation of rapid clearance of STEC in stool specimens among azithromycin-treated patients and the high rate of long-term STEC carriage in the control group led to the decision to provide azithromycin treatment for 15 patients with remaining symptoms, associated with curtailing of social or work life. The patients had a history of STEC carriage between 30 and 63 days prior to start of decolonization therapy. Ten of these 15 long-term carriers had been part of the initially nontreated control group (Figure 2). Of the other 5 patients, 4 had previously been treated at district hospitals or by general practitioners and were then referred to our university outpatient clinic for azithromycin treatment because of persistent pathogen shedding. One asymptomatic long-term carrier was identified by public health authorities. Decolonization therapy was offered to these patients because of individual restrictions in their social or work life. After completion of treatment, all patients had at least 3 STEC-negative stool specimens, indicating successful STEC decolonization. There were no signs of HUS induction due to azithromycin therapy as determined by unchanged serum creatinine and lactate dehydrogenase levels as well as unchanged platelet counts (TABLE 3).

Table 1. Patient Characteristics

Characteristics	Azithromycin (n = 22)	Control (n = 43)	*P* Value[a]
Age, mean (SD), y	43.7 (14.7)	48.3 (21.9)	.31
Male sex, No. (%)	7 (31.8)	15 (34.9)	.97
Hemolytic uremic syndrome, No. (%)	22 (100)	15 (34.9)	<.001
Duration of investigation, mean (SD), d[b]	40.7 (6.6)	44.9 (11.8)	.13
Samples investigated, mean (SD)	6.6 (1.9)	6.8 (3.2)	.66
Duration confirmed positive, mean (SD), d[c]	8.9 (6.3)	34.3 (19.3)	<.001
Time to treatment, mean (SD), d[d]	11.8 (3.2)		

[a] By Mann-Whitney test for age, duration of investigation, samples investigated, and duration confirmed positive; by χ^2 test for sex and hemolytic uremic syndrome.
[b] Time from onset of clinical symptoms to last available culture result.
[c] Time from onset of clinical symptoms to last positive culture result.
[d] Time from onset of clinical symptoms to start of azithromycin treatment.

The remaining 15 patients in the initially nontreated control group who remained STEC-positive at day 42 and were not yet decolonized at the time of data analysis had different outcomes: 4 patients were lost to follow-up after days 78, 73, 67, and 50 without a confirmed negative status. Four patients showed late spontaneous conversion to confirmed noncarrier status between days 43 and 68. Seven patients accepted azithromycin therapy and were all rapidly decolonized without any clinical sequelae. This post hoc decolonization group included 2 patients with documented STEC O104:H4–positive stool specimens at the end of October 2011, indicating STEC carriage for more than 160 days. Even among these long-term carriers, stool samples were negative for STEC after eradication therapy. During 6 months of follow-up, as of December 20, 2011, no patient in either group required rehospitalization or experienced a novel HUS episode.

COMMENT

Antibiotic treatment is controversial in STEC infection,[8] and most recommendations discourage antibiotic use to avoid induction of Shiga toxin expression with the potential risk of developing HUS[14]; however, there is still no clear evidence for increased risk of HUS due to antibiotic treatment.[15] The STEC strain of the current German outbreak was characterized as *E coli* harboring a Shiga toxin–encoding phage in the genetic background of EAggEC.[2,3]

Enteroaggregative *E coli* plays a major role in traveler's diarrhea, for which fluoroquinolones, azithromycin, and rifaximin are recommended therapy.[6] Because of the potential of induction of Shiga toxin expression, fluoroquinolones[16-18] are not suitable for therapy of enteroaggregative STEC infection. For rifaximin, limited experience is available on invasive disease and its use is not recommended in complicated diarrhea with systemic toxicity, fever, or bloody stools.[19] Azithromycin, in contrast, was demonstrated to suppress Shiga toxin expression in the majority of investigated STEC.[17,18,20] Moreover, the eradication rate reported in EAggEC infections is 86%.[21] Therefore, azithromycin might be considered a potentially effective and safe antibiotic for the treatment of enteroaggregative STEC infection.

In patients receiving eculizumab therapy, prophylaxis of meningitis was performed using oral azithromycin for 14 days in addition to vaccination. Following this regimen, the duration of STEC shedding was compared with STEC shedding in patients receiving no antibiotic treatment covering *E coli*. In the initial azithromycin group, the duration of carriage was significantly lower compared with untreated patients, and all azithromycin-treated patients remained STEC-negative after the end of the 14-day treatment period.

Withholding azithromycin treatment, in contrast, resulted in a high rate of long-term carriage. Thus, STEC shedding was documented for more than 4 weeks after onset of clinical symptoms in more than

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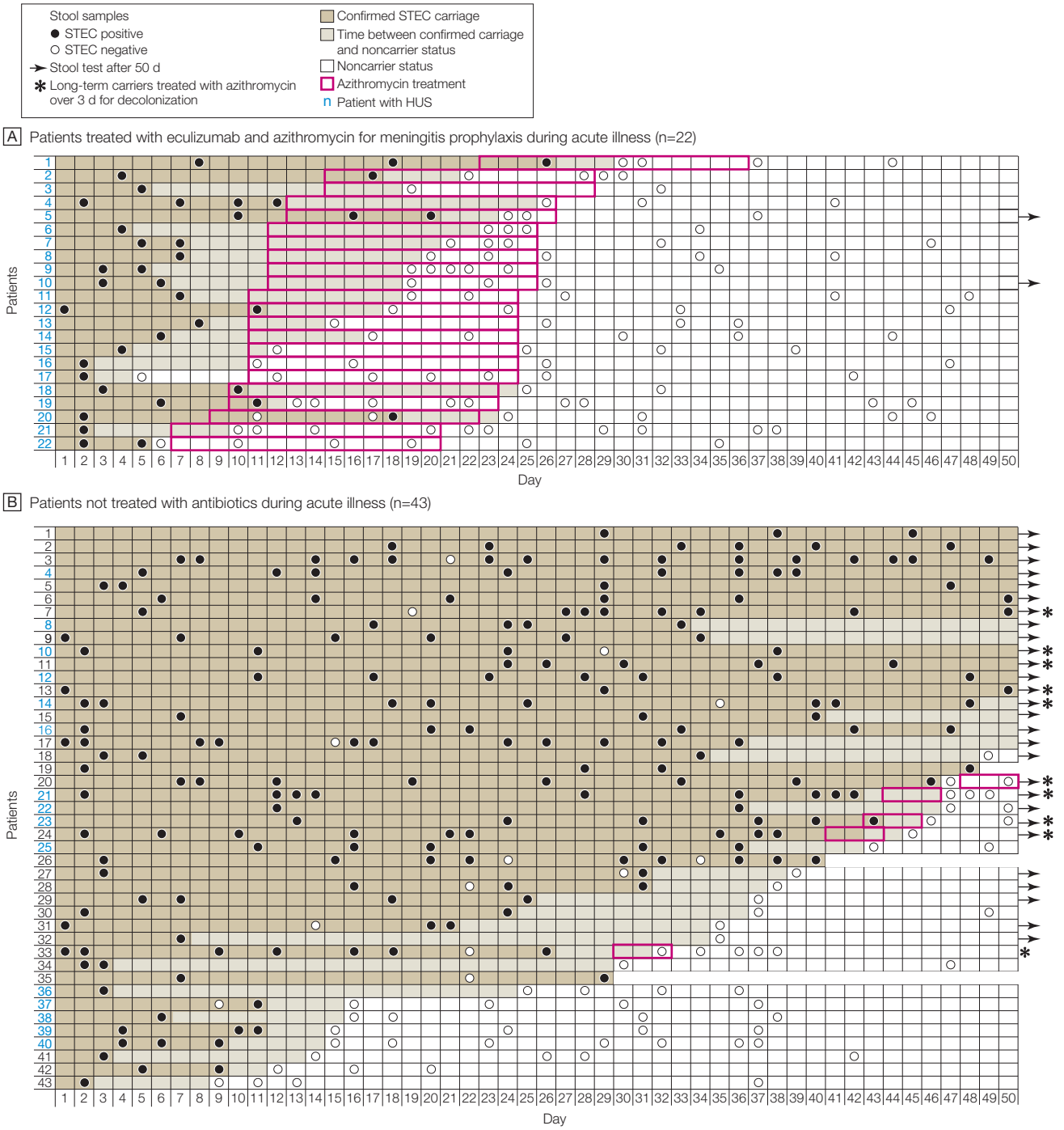
two-thirds of patients. Some patients remained STEC-positive for more than 160 days. The high rate of long-term carriage observed in patients not treated with antibiotics in our study suggests that hundreds of patients still carry STEC O104:H4 in Germany. Despite this observation, the outbreak ended on July 4, 2011, without significant numbers of follow-up cases,[1] suggesting a low potential for person-to-person transmission of the current outbreak strain.

In the control group, no statistical differences concerning duration of carriage were found between patients with and

Figure 2. Time Course of STEC O104:H4 Shedding



STEC indicates Shiga toxin–producing enteroaggregative *Escherichia coli*.

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without HUS. These data suggest that the course and severity of the disease itself are not associated with duration of carriage.

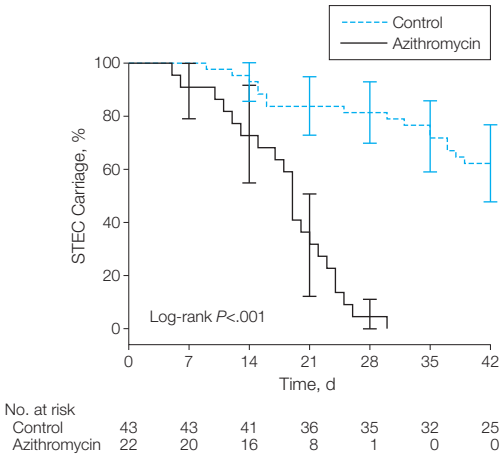
The STEC strain is clonally related to a previously described strain (HUSEC041),[22] containing a Shiga toxin variant (stx2a) and several virulence genes encoded on the EAggEC virulence plasmid. Part of the EAggEC pathogenicity seems to be its ability to produce a strong biofilm by enhancing mucus secretion of the intestinal mucosa with trapping of the bacteria, probably resulting in persistent colonization.[9,10,23,24]

Patients with STEC are considered infective as long as bacteria can be detected in the stool. The infectious prevention law of the German health authorities restricts long-term carriers regarding their social and work life (eg, food processing and health services jobs), which represents a high individual socioeconomic burden. Clinically, long-term carriage might cause persistent diarrheal symptoms.[9,25] Therefore, decolonization is an important need for affected patients.

In this study, we found no evidence that azithromycin worsened the clinical course with regard to local or systemic symptoms in patients with severe HUS. Additionally, partial immunity against Shiga toxin is considered in persons chronically colonized or recurrently infected with STEC.[26] Among the 15 asymptomatic long-term STEC carriers treated with oral azithromycin for 3 days, all patients became STEC-negative within a few days. These data yield a higher eradication rate of the outbreak strain compared with azithromycin in acute EAggEC diarrhea.[21] Among long-term carriers receiving decolonization therapy, no clinical or laboratory measures indicated induction of HUS, corroborating our finding suggesting that oral azithromycin most likely may be used safely for decolonization of STEC O104:H4 long-term carriage. Additionally, as all patients remained STEC-negative after azithromycin therapy, no development of resistance against azithromycin was observed.

Our study had several limitations. The study was limited to patients treated at a single site and the sample size is relatively small. The use of eculizumab and concomitant azithromycin prophylaxis was not based on randomized assignment. Due to the unexpected outbreak onset, stool sampling was initially scheduled following clinical decision but not according to a prespecified protocol. Therefore, several outpatient STEC carriers may have been missed; moreover, HUS patients are overrepresented because patients with a severe clinical course of STEC infection were prefer-

Figure 3. Course of STEC Carriage After Onset of Clinical Symptoms in Patients Receiving Azithromycin and Untreated Patients



STEC indicates Shiga toxin–producing enteroaggregative *Escherichia coli*. Error bars indicate 95% CIs.

Table 2. Characteristics of HUS and Non-HUS Patients in the Untreated Group (n = 43)

Characteristics	HUS (n = 15)	Non-HUS (n = 28)	*P* Value[a]
Age, mean (SD), y	42.8 (23.0)	51.3 (21.1)	.31
Male sex, No. (%)	5 (33.3)	10 (35.7)	.86
Duration of investigation, mean (SD), d[b]	43.7 (8.4)	45.6 (13.4)	.83
Duration confirmed positive, mean (SD), d[c]	32.4 (19.2)	35.3 (19.6)	.98

Abbreviation: HUS, hemolytic uremic syndrome.
[a] By Mann-Whitney test for age, duration of investigation, and duration confirmed positive; by χ^2 test for sex.
[b] Time from onset of clinical symptoms to last available culture result.
[c] Time from onset of clinical symptoms to last positive culture result.

Table 3. Characteristics of 15 Patients Receiving Decolonization Therapy as Proof of Principle

Characteristics	Value		
Age, mean (SD), y	51.7 (19.1)		
Male sex, No. (%)	2 (13.3)		
Duration confirmed positive before therapy, mean (SD), d[a]	48.3 (10.3)		
Time to decolonization treatment, mean (SD), d[b]	49.1 (10.8)		
Time to confirmed negative results, mean (SD), d[c]	52.8 (10.7)		
Hemolytic uremic syndrome–diagnosing measures, mean (SD)	Before Therapy	After Therapy	*P* Value[d]
Lactate dehydrogenase, U/L	172.1 (41.1)	172.9 (34.2)	.87
Creatinine, mg/dL	0.78 (0.10)	0.76 (0.10)	.06
Thrombocytes, cells/nL	262.9 (71.8)	256.8 (68.3)	.60

SI conversion factor: To convert creatinine to μmol/L, multiply values by 88.4.
[a] Time from onset of clinical symptoms to last positive culture result prior to therapy.
[b] Time from onset of clinical symptoms to start of azithromycin treatment.
[c] Time from onset of clinical symptoms to first confirmed negative stool sample.
[d] By paired *t* test.

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ably admitted to our university hospital. However, all patients were treated by the same team of physicians, resulting in stringent and homogenous procedures.

Our findings suggest that among patients with STEC infection, exposure to azithromycin is associated with a lower frequency of long-term STEC carriage and that among long-term carriers of STEC, azithromycin given for 3 days was associated with decolonization. These findings warrant confirmation for other STEC strains, as well as prospective evaluation and possible clinical trials.

Author Contributions: Drs Nitschke, Sayk, and Knobloch had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Nitschke and Sayk contributed equally.
Study concept and design: Nitschke, Sayk, Steinhoff, Derad, Wellhöner, Büning, Katalinic, Lehnert, Solbach, Knobloch.
Acquisition of data: Nitschke, Sayk, Härtel, Roseland, Hauswaldt, Steinhoff, Fellermann, Derad, Wellhöner, Büning, Tiemer, Lehnert, Knobloch.
Analysis and interpretation of data: Nitschke, Sayk, Härtel, Roseland, Hauswaldt, Steinhoff, Derad, Katalinic, Rupp, Lehnert, Solbach, Knobloch.
Drafting of the manuscript: Nitschke, Sayk, Hauswaldt, Steinhoff, Katalinic, Knobloch.
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Administrative, technical, or material support: Härtel, Roseland, Hauswaldt, Steinhoff, Fellermann, Wellhöner, Tiemer, Rupp.
Study supervision: Nitschke, Sayk, Steinhoff, Wellhöner, Büning, Lehnert, Solbach, Knobloch.
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