The Isolation Rate of Pseudomonas aeruginosa Opportunistic Pathogen and their Antimicrobial Responses in HIV-1 Positive and Negative Diarrhoea Patients at North-West Part of Ethiopia

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Abstract

**Background:** Pseudomonas aeruginosa is an opportunistic pathogen in immune-compromised patients. However, reports of infections of Pseudomonas aeruginosa were limited in patients infected with HIV. The aim of the study was to determine the frequency of isolation and sensitivity pattern of P. aeruginosa against the commonly used antibiotics in HIV-1 positive and negative diarrheic patients.

**Methods:** Laboratory based cross-sectional study of 312 (199 HIV seropositive and 113 HIV seronegative) diarrheic patients of both genders were investigated at University of Gondar Hospital, Gondar, Ethiopia. P. aeruginosa isolates were isolated from diarrheic stool and identified as per standard methods and tested for antimicrobial susceptibility.

**Result:** Pseudomonas aeruginosa was isolated from 29.5% (92/312) of diarrheic patients, of which 20.5% were from HIV positive subjects. Out of 58 H. zoster positive diarrhoea patients, 47 (81%) were HIV positive and 11 (19%) were HIV seronegative (P < 0.002). The proportion of patients with weight loss and P. aeruginosa infection was significantly higher in those with HIV co-infection (P < 0.05). Among P. aeruginosa isolates 96.4%, 78.6%, 73.2%, 71.4%, 68.8% and 62.5% were sensitive to polymyxin B, gentamycin, norfloxacin, kanamycin, ciprofloxacin and nalidixic acid, respectively. The highest resistance observed was against ampicillin (96.4%), tetracycline (92%), trimethoprim-sulphamethoxazole (89.3%) and chloramphenicol (74.1%). One hundred nine (97.3%) out of the 112 P. aeruginosa isolates were found to be multiple drug resistant.

**Conclusion:** Though high proportion of P. aeruginosa strains were reported in HIV seropositive diarrheic patients, detailed investigation is important to get comprehensive information for better treatment of diarrhoea in HIV/AIDS patients. In this study, ampicillin, tetracycline, trimethoprim-sulphamethoxazole and chloramphenicol may not be used for the treatment of P. aeruginosa infection, unless culture and sensitivity tests are done prior to treatment. Effective public health measures such as prevention of infections through quality environmental sanitation and personal hygiene should be emphasized.

**Keywords:** P. aeruginosa; HIV-1; Diarrhea; Prevalence; Antimicrobial sensitivity

**Introduction**

HIV/AIDS pandemic is still extremely dynamic and growing worldwide. In the world, about 33.2 million people are estimated to be infected with HIV/AIDS [1]. From this more than 22.5 million are expected to live in Sub-Saharan Africa [1]. HIV is now the leading cause of death worldwide among the age group of 15-24. Among the most heavily HIV/AIDS affected countries, Ethiopia is the third next to India and South Africa in 2005 [2]. The prevalence of HIV infection in Ethiopia is currently over 3.5% [3]. HIV, in Ethiopia, is mostly associated with opportunistic infections such as candidiasis, tuberculosis, CNS mass lesion [4] and diarrhea [5].

Diarrheal diseases are one of the major causes of morbidity and mortality in developing countries [6]. The situation is severe in sub-Saharan Africa, a region where prevalence of HIV is high [7]. Diarrhea, the passage of loose or watery stools at least three times in 24 hours, is one of the clinical manifestations of HIV infection and usually tends to be chronic [6]. Chronic diarrhea, an episode that begins acutely and lasts for more than four weeks [6], in tropical countries is associated with weight loss and is often the presenting illness of HIV infected individuals. This diarrhea wasting syndrome in association with a positive HIV serology test is an AIDS defining illness in the World Health Organization’s classification [8]. Diarrhoea occurs in up to 70% of the patients infected with HIV in Africa [9]. In Ethiopia, as in other sub-Saharan Africa, morbidities from diarrhoeal diseases [6] and HIV/AIDS [10,11] are serious health problems. Of those bacterial infections, P. aeruginosa is one of the opportunistic bacterium in HIV/AIDS patients with diarrhea [12].

Pseudomonas aeruginosa is a Gram-negative, aerobic rod belonging to the bacterial family Pseudomonadaceae. P. aeruginosa is an opportunistic pathogen, meaning that it exploits when there is some break in the host defense to initiate an infection [13]. It causes different infections such as, urinary tract, respiratory system, soft tissue, bone and joint, gastrointestinal and a variety of systemic infections, dermatitis and bacteremia particularly in patients with severe burns, cancer and AIDS patients [14]. One characteristic of P. aeruginosa infections in...
HIV-infected population is the predominance of community-acquired rather than nosocomial cases [15-17].

Diagnosis of *P. aeruginosa* infection depends up on isolation and laboratory identification of the bacterium. It grows well on most laboratory media. It is identified on the basis of its Gram morphology, inability to ferment lactose, positive oxidase reaction, its fruity odor and ability to grow at 42°C [18]. Fluorescence under ultraviolet light is helpful for early identification of *P. aeruginosa* colonies [19,20].

*Pseudomonas aeruginosa* is notorious for its resistance to antibiotics and is therefore, a particularly dangerous and dreaded pathogen [21,22]. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharides (LPS). Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics [22]. Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally occurring antibiotics [23]. Moreover, *P. aeruginosa* maintains antibiotics resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of transduction and conjugation [24]. Generally, *P. aeruginosa* develops resistance to nearly any antimicrobial agent. Resistance is problematic at three levels: intrinsic resistance, acquired resistance and emergence resistance during therapy [25]. *P. aeruginosa* is frequently resistant to many commonly used antibiotics. Each of them must be considered when choosing an antibiotic regimen for patients infected with *P. aeruginosa* [26-28].

In Ethiopia, as far as my exploration is concerned, there are no studies conducted on the prevalence and sensitivity pattern of *P. aeruginosa* against the commonly used antibiotics in diarrheic patients with and without HIV infection. Therefore, the objective of this study is to determine the frequency of isolation and sensitivity pattern of *P. aeruginosa* against the commonly used antibiotics in HIV-1 positive and negative diarrheic patients. The outcome of this study may help as baseline information for empirical treatment of HIV 1 positive diarrheic patients affected by *P. aeruginosa*.

Materials and Methods

Study design and subjects

In this cross-sectional study, consecutive diarrheic patients diagnosed at the outpatient department of the University of Gondar Hospital, in Gondar, Ethiopia were included. Informed consent was obtained from all subjects and the study was approved by the Research Ethics Committee of the University.

Chronic diarrhea was defined as an average of three or looser watery bowl movement per day for at least two weeks before the study [29]. Acute diarrhea was defined as an episode that lasted less than 14 days [29]. All subjects underwent physical examination. Socio-demographic and clinical data were recorded by medical doctors on structured and pre-tested questionnaires.

The sample size

The sample size for the study was calculated by considering a 95% level of confidence, 90% proportion of the study done in Addis Ababa [30] and Jimma [31] respectively, 5% margin and 20% contingency. As the result, 312 patients with diarrhea were included in the study, out of which 199 were HIV seropositive and 113 HIV seronegative.

HIV serology

After obtaining informed consent and appropriate pre-test counseling, about 5 ml of venous blood was taken from each subject and sera were separated by centrifugation and stored at -20°C until used. The presence of HIV antibodies was determined by an enzyme linked immunosorbent assay following the manufacturer’s instruction (Vironostica HIV Uni-Form II plus O, Organon Teknika, Boxtel, the Netherlands) [32].

Stool collection and isolation of pathogens

Diarrheic stool specimens were collected as fresh in sterile containers and processed immediately after collection following standard procedures [33]. The specimens were cultured on the MacConkey agar (BBL). All the inoculated plates of McConkey agar were incubated at 42°C for 48 hrs.

The *P. aeruginosa* isolates were identified based on colony characteristics, gram staining methods and by various biochemical tests as given below. In brief, they were identified on the basis of their Gram morphology, inability to ferment lactose, oxidase and hydrogen peroxide reaction, its fruity odor and its ability to grow at 42°C [34]. A single colony of non-lactose fermenting type from MacConkey plates was aseptically picked and inoculated in about 3 ml of nutrient broth and incubated at 35-37°C for 2-4 hours until growth was ascertained by turbidity. From this broth culture, identification of *P. aeruginosa* was performed with the help of biochemical tests which routinely included triple sugar iron (TSI) agar slant, lysine iron (LI) agar slant, Urea agar slant, Simmon’s citrate agar slant, Shahidi Indole Motility (SIM) medium, Mannitol broth (1%) and glucose broth (1%). Biochemical results were interpreted by using Bergey’s manual [35].

Antimicrobial sensitivity testing

Susceptibility testing of all strains was done on Muller-Hinton agar with commercial antibiotic discs using Kirby-Bauer method [36]. Each isolate was sub-cultured on a plate of MacConkey agar and incubated at 37°C for 18 to 24 hours. From a pure culture of each isolate 4 to 5 single colonies were randomly selected and transferred to a test tube of Trypticase Soy broth by touching the top of each colony with a sterilized wire loop [34]. Trypticase Soy broth incubated at 37°C for 8 hours to obtain moderate turbidity. The suspension (a portion of it) was diluted with a sterile saline to obtain turbidity visually equal to that of the standard of McFarland 2 [37].

Susceptibility testing of all strains against ampicillin (Amp) 10 µg; chloramphenicol (Chl) 30 µg; gentamicin (Gen) 10 µg; nalidixic acid (Nal) 30 µg; polymyxin B (Pol) 30 µg; norfloxacin (Nor) 30 µg; tetracycline (Tet) 30 µg; kanamycin (Kan) 30 µg; ciprofloxacin (Cip) 5 µg and trimethoprim-sulphamethoxazole (Sxt) 25 µg were carried out using standard method (Andualem and Geyid, 2003). *Staphylococcus aureus* (ATCC 27813) and *Escherichia coli* (ATCC5922) strains susceptible to all the antibiotics tested were used as control.

Finally, diameters of inhibition zones were measured in millimeters using a sliding caliper, which is held on the back of the inverted plate. The sizes of the zones of inhibition were interpreted by using zone diameter interpretive standards [38]. The intermediate readings were considered as sensitive for a purpose of assessment of the data. Multiple drug resistance was defined as a given bacterium which is resistant to more than one antibiotic.
Statistical analyses

Data were analyzed using SPSS version 16 statistical package. Comparisons *P. aeruginosa* positive among HIV patients with diarrhoea were made using both Chi-square test at significance level *P* < 0.05 and compared means (student t test; independent samples test). *P* values less than 0.05 were considered statistically significant.

### Results

Table 1 shows socio-demographic and clinical features of the patients by HIV serostatus. A total of 312 diarrheic patients were included in the study. Of this, one hundred eighty (57.7%) were males and 132 (42.3%) were females. Their mean age was 29 (range 15 to 67 years). A significant number of the patients were in the age group of 25 to 44 years. On the age group 15-24 HIV seropositive diarrhoea patients, the proportion of males and females was 3.5% to 4.5%, respectively. It was also observed that the proportion of males and females within the age groups greater than 34 years were 14.7% and 8.3%, respectively (*P* < 0.043). The prevalence of HIV in diarrhoea patients with regard to marital status (*P* < 0.01) and occupation distribution (*P* < 0.01) has shown significant variation in the study subjects. Of the diarrhoea patients, 66 (21.2%) government employees, 53 (17.0%) farmers, 48 (15.3%) students, 42 (13.4%) house wives, and 103 (33%) others were found to be HIV positive. Acute and chronic diarrhea was diagnosed in 46.5% and 53.5% of the patients, respectively. Among patients having acute diarrhoea, 79 (25.5%) and 66 (21.2%) were HIV positive and negative, respectively. In the case of patients having chronic diarrhoea, 59 (18.9%) and 108 (34.6%) were HIV positive and negative, respectively. The prevalence of HIV infection in the patients was 63.8% (199/312).

In this investigation, the association of the history of *Herpes zoster* in HIV seropositive and negative diarrhoea patients was seen. Out of 199 *H. zoster* positive diarrhoea patients, 161 (80.9%) were HIV positive and 38 (19.1%) were HIV seronegative (*P* < 0.002) (Table 1). Intestinal parasites were detected in 61 (19.6%) and 38 (12.2%) of HIV negative and HIV positive patients out of the total (312) diarrheic patients, respectively (*P* < 0.002).

| Characteristics | HIV serostatus | Total No. (%) | *P*-value |
|-----------------|---------------|---------------|-----------|
|                 | HIV positive (%) | HIV negative (%) | |
| HIV positive No. (%) | HIV negative No. (%) | Total No. (%) |
| **P. aeruginosa isolates** | 64 (32.2) | 135 (67.8) | 28 (24.8) | 85 (75.2) | 312 (100) | 0.004 |
| **Nature of diarrhea** | 31 (15.8) | 58 (29.1) | 10 (8.8) | 35 (31) | 134 (42.9) | 0.514 |
| Watery | 21 (10.6) | 35 (17.6) | 8 (7.1) | 31 (27.4) | 95 (30.4) | |
| Bloody | 12 (6) | 42 (21.1) | 10 (8.8) | 19 (16.8) | 83 (26.6) | |
| Mucoid | 66 (21.2%) | 135 (67.8%) | 28 (24.8%) | 85 (75.2%) | 312 (100%) | |
| **Weight loss** | 61 (30.7) | 119 (59.8) | 12 (10.6) | 27 (23.9) | 219 (70.20) | 0.0001 |
| Yes | 3 (1.5) | 16 (8) | 16 (14.2) | 58 (51.3) | 93 (29.8) | |
| No | 64 (32.2) | 135 (67.8) | 28 (24.8) | 85 (75.2) | 312 (100) | 0.004 |
| Total | 12 (6) | 42 (21.1) | 10 (8.8) | 19 (16.8) | 83 (26.6) | |
| **Abdominal cramp** | 25 (12.6) | 35 (17.6) | 12 (10.6) | 26 (23) | 98 (31.4) | 0.218 |
| Yes | 39 (4.5) | 100 (50.3) | 16 (14.2) | 59 (52.2) | 214 (88.6) | |
| No | 64 (32.2) | 135 (67.8) | 28 (24.8) | 85 (75.2) | 312 (100) | 0.004 |
| Total | 15 (7.5) | 18 (9) | 8 (7.1) | 16 (14.2) | 57 (18.3) | |
| **Tenesmus** | 49 (24.6) | 117 (58.8) | 20 (17.7) | 69 (61.1) | 255 (81.7) | |
| Yes | 64 (32.2) | 135 (67.8) | 28 (24.8) | 85 (75.2) | 312 (100) | 0.004 |
| No | 54 (27.1) | 124 (62.3) | 23 (20.4) | 72 (63.7) | 273 (87.5) | |
| Total | 47 (29.2) | 114 (70.8) | 28 (24.8) | 85 (75.2) | 312 (100) | 0.001 |

Compared means (student t test; independent samples test) was used at *P* values less than 0.05.

Table 2: Clinical symptoms and diarrhea status in association with that of *P. aeruginosa* isolates in HIV seropositive and negative diarrhoea patients, University of Gondar Hospital, 2003/4.
The prevalence of *P. aeruginosa* isolates in HIV positive and negative subjects was investigated (Table 2). The most important finding of this study was that out of the total diarrhoea patients, 92 (29.5%) was *P. aeruginosa* positive in both HIV positive and negative study subjects. Out of the total *P. aeruginosa* isolates, 64 (20.5%) were isolated from HIV positive and 28 (9%) from HIV negative diarrhoea patients (P < 0.05). Of the total 199 (100%) *H. zoster* infected diarrheic patients, 161 (80.9%) and 38 (19.1%) were HIV positive and negative, respectively. From both HIV and *P. aeruginosa* positive study subjects (64), 47 (73.4%) were positive for *H. zoster* while the rest 17 (26.6%) negative for *H. zoster* (P < 0.05) (Table 2).

Clinical symptoms and diarrhoea status in association with that of *P. aeruginosa* isolates in HIV seropositive and negative diarrhoea patients is presented in Table 2. In this study, nature of diarrhoea and abdominal cramp of such diarrhoea were not statistically associated with *P. aeruginosa* isolates (P > 0.05). On the other hand, marked weight loss was observed in 32.2% and 24.8% of patients with HIV seropositive and HIV seronegative, respectively. The proportion of patients with weight loss and *P. aeruginosa* infection was significantly higher in those with HIV co-infection (P < 0.05). Based on the independent samples test analysis of HIV serostatus result, weight loss, and parasites as independent variables and *P. aeruginosa* as dependent variable, has been shown significant association (P < 0.05) between the parameters and prevalence of *P. aeruginosa*.

Antimicrobial sensitivity test of *P. aeruginosa* isolates against the chosen 10 antimicrobial agents are presented on Figure 1. All the strains were not 100% sensitive to any of the chosen antibiotic agents but 96.4%, 78.6%, 73.2%, 71.4%, 68.8% and 62.5% of *P. aeruginosa* strains were sensitive to Pol, Gen, Nor, Kan, Cip and Na, respectively. The highest resistance pattern was observed against chloramphenicol (74.1%), Sxt (89.3%), Tet (92%) and Amp (96.4%).

Resistance against one or more antibiotics is shown in Table 3. Out of the 112 *P. aeruginosa* strains, 109 (97.3%) showed multiple resistance. In all, 36 different patterns of resistance were noted. The frequency of Amp, Sxt, Na, Nor, Gn, Kn, Ch, Ttc, Cip, and Amp, Sxt, Ch, Tet pattern of resistance are 19 (17%) and 24 (25%), respectively, which are the highest in comparison with other pattern of resistance. Three and five pattern of resistance were observed in 16 (14.3%) and 17 (15.3%) *P. aeruginosa* isolates, respectively. *Pseudomonas aeruginosa* isolates with greater than three pattern of antimicrobial resistance account for 89 (79.6%). In this finding, there is no any strain, which is sensitive, or resistance against to all antimicrobial agents.

**Discussion**

HIV infection has become the dominant health problem in many parts of sub-Saharan Africa, including Ethiopia [39]. The result of this study in terms of HIV infection agrees with that indicated on the 2005 UNAIDS report [31]. It was observed that the highest infection rates are concentrated in the 15-34 year age group. Within this age group, prevalence among females was found to be greater than that of males. Other than gender, marital status and occupation have also shown impact on the distribution of HIV. The association of *H. zoster* infection and HIV was also found to be significantly (p < 0.05) high 161/199 (80.9%). *H. zoster* infection was one of the most common HIV-related opportunistic infections and diseases in HIV seropositive patients [40,41]. According to this study, the infection rate of *H. zoster* in both HIV and *P. aeruginosa* positive was 73.4%. This is may be due to weak immune response of HIV positive study subjects. As well known, both *H. zoster* [40,41] and *P. aeruginosa* [15] infections were the most common opportunistic infections in HIV positive individuals.

One of the major manifestations of the HIV disease in the region is the diarrhea [42,43]. In this investigation, chronic diarrhoea was found to be more common than acute diarrhoea in HIV positive subjects. On the other hand, acute diarrhoea was more common than chronic diarrhoea in HIV negative subjects.

Intestinal parasitism occurs widely throughout Ethiopia [44]. It was reported that intestinal parasites were one of the causative agents of diarrhoea in HIV seropositive patients [5,45]. In line with these reports; we found a high prevalence (19.6%) of intestinal parasites in HIV seropositive diarrheic patients included in the present study. Earlier data suggest that intestinal parasite infections can affect the nutritional status of infected people through modification of food intake, digestion and absorption capacity [46]. Acquired immunodeficiency syndrome (AIDS) caused by HIV infection predisposes individuals to several diarrheagenic bacterial diseases of various species [47] in addition to several viral, protozoal and parasitic etiological agents [48]. This study was designed to investigate the prevalence and antimicrobial responses of *P. aeruginosa* isolated from HIV seropositive and negative diarrheic patients.

*Pseudomonas aeruginosa* is important opportunistic pathogens of bacteria in HIV-infected host [15]. This study showed a high prevalence of *P. aeruginosa* in diarrheic patients with or without HIV co-infection in Northwest Ethiopia (29.5%). In this investigation, the prevalence of
the isolation of *P. aeruginosa* in HIV seropositive subjects was 64/199 (20.5%), which was lower than 41/135 (30.7%) isolates from HIV-infected patients [49]. However, the result of this study was almost the same with the report of (19.2%) Osazuwa et al. [50], in Benin but higher than the isolated (8.7%) and (4.7%) strains reported by Olayinka et al. [51] and Hernandez et al. [52], respectively. Even the isolation rate is high in some studies [51,52], *P. aeruginosa* infection is not frequent bacterial disease in highly immunodeficient HIV-infected patients. It may be fatal in the advanced stages of HIV diseases, especially when patients have taken the common and cheap available antibiotics [53] and the society with poor hygiene conditions. The incidence of *P. aeruginosa* infections in HIV/AIDS patients appears to be on the rise [54].

The results of this study revealed that the prevalent rate of *P. aeruginosa* is high in HIV seropositive diarrheic patients. In this finding, HIV positive patients without *P. aeruginosa* were also affected by diarrhoea. This shows that diarrhoea may be associated with other diarrhoeagenic agents or mechanisms rather than *P. aeruginosa* [55]. Then, detail investigation has to be made in order to get comprehensive information for better treatment of diarrhoea in HIV/AIDS patients.

In this study, all the isolates were resistant to the cheap, commonly available antibiotics, such as Tet, Sxt and Chl in comparison with that of expensive and unavailable antibiotics such as Pol, Gen, Nor and Cip. Even Kan and Nat although shows some in vitro activities in this study, they are equally ineffective due to their pharmacodynamic or pharmacokinetic characteristics [56]. However, the susceptibility patterns of *P. aeruginosa* vary geographically, and susceptibility tests should be done as an alternative means to select antimicrobial therapy [57]. According to this finding, Nor, Gen, Pol and Cip may be used as drug choice for empirical treatment. On the other hand, Amp, Tet, Sxt and Chl may not be used as the drugs of choice for the treatment of *P. aeruginosa* infection unless culture and sensitivity tests are done prior to treatment. The high prevalence of multidrug resistant indicates a serious need for broad-based, local antimicrobial resistance surveillance for continuous tracking of antibiotic resistance trends among all clinical relevant isolates and introduction of effective interventions to reduce multidrug resistance in such pathogens [58-60].

The occurrence of resistant strains in this study may be the spread use of these drugs. This may cause selective pressure of resistance problems on the enteric bacteria as a whole circulating in the community [61].

Culture and drug sensitivity results generally determine the choice of antibiotic for enteric bacterial infections. The choice of antimicrobial agents should be dictated by local resistance patterns and the patients' history antibiotic exposure [62,63]. Regardless of the choice of antibiotics, doses should be selected to maximize serum levels for enhanced activity. Combination therapy is also recommended to prevent the emergency of resistant strains. However, the effectiveness of combination therapy in preventing the emergency of antibiotic resistance in *P. aeruginosa* infections remains controversial. According to Carmeli et al. [64], combination therapy was not associated with a disease in the emergency of resistance. On the other hand, Jacobson et al. [65] found that combination therapy was associated with less frequent development of resistance than mono-therapy.

In this study, high prevalence of *P. aeruginosa* was observed in diarrheic patients with HIV infection suggesting that there is high human fecal contamination. This calls for efforts for strengthening control programs such as quality sanitation at community level to reduce risk factors of acquiring this diarrhoeagenic bacterium. In addition, routine examination of stool for *P. aeruginosa* with sensitivity test could significantly benefit the HIV infected and uninfected individuals in reducing morbidity and improving quality of life. This study was the first of its kind on the prevalence and antimicrobial responses of *P. aeruginosa* in HIV-1 positive and negative diarrhoea patients in Ethiopia. Therefore, the information obtained in this study is significant for empirical treatment of diarrheic patients’ affected *P. aeruginosa*.

In this finding, Amp, Tet, Sxt and Chl may not be used for the treatment of *P. aeruginosa* infection, unless culture and sensitivity tests are done prior to treatment. In underdeveloped countries like Ethiopia, besides limiting therapeutic options and the high cost of alternative effective agents, resistant organisms may lead to longer hospitalization and an increased risk of death. The development of new antimicrobial agents may offer short-term solution to this problem but in the long run more effective measures such as health education and further research on the prevention of infections through quality sanitation, vaccination and immunization should be emphasized. Based on this study, I would recommend that public health measures should continue to emphasize the importance of environmental and personal hygiene.

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