

# Concern for medical students due to their cell phones' comparatively high contamination with *Pantoea agglomerans* bacteria with reduced sensitivity to some antimicrobials

Ahmed E. Taha

Microbiology and Immunology Unit, Department of Pathology, College of Medicine, Jouf University, Sakaka, Saudi Arabia

## ABSTRACT

The significance of *Pantoea agglomerans* bacteria in diseases linked to healthcare is underappreciated due to a shortage of information on their spread. This is the first study in Saudi Arabia to examine the possible contribution of medical students' cell phones (CPs) to the transmission of *P. agglomerans* to hospitalized patients and to evaluate their antibiotic susceptibility profiles. In total, 250 CPs were swabbed. *P. agglomerans* was isolated and identified using standard techniques. The suspected colonies

were confirmed by the Vitek 2 compact system. The isolates' antimicrobial susceptibility profiles were assessed using Epsilon assays, and the results were interpreted according to Clinical and Laboratory Standards Institute guidelines. The frequency of *P. agglomerans* contamination of CPs was found to be relatively high (20.40%; 51 isolates/250 samples). Many isolates showed varying degrees of reduced sensitivity to ampicillin, aztreonam, cefazolin, cefotaxime, cefuroxime, and ertapenem antibiotics. To implement optimal infection prevention and control policies regarding the possibility of antibiotic-resistant *P. agglomerans* transmission through medical students' contact points with hospitalized patients during their frequent activities in healthcare settings, health policymakers may find value in utilizing this study's results.

Correspondence: Ahmed E. Taha, Microbiology and Immunology Unit, Department of Pathology, College of Medicine, Jouf University, Sakaka 72388, Saudi Arabia.  
E-mail: aeattia@ju.edu.sa - drahmadmicro@yahoo.com

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## Introduction

Smart devices are widely used all over the world due to the multiple benefits of their variable applications, such as rapid communication and access, especially among students, teaching staff, and healthcare providers. Cell phones (CPs) facilitate the education process, enable distance electronic learning, and reduce the need for paper-based resources.<sup>1,2</sup> CPs of medical personnel can be rapidly and easily contaminated by microorganisms from patients, medical devices, and medical environment such as bacteria,<sup>3</sup> and fungi.<sup>4</sup> Recently, the rate of healthcare-associated infections (HAIs) has increased especially among the immunocompromised patients.<sup>5</sup>

Members of the genus *Pantoea* belong to the *Enterobacteriaceae* family and are Gram-negative, non-spore-forming, non-capsulated bacilli of environmental nature that can be isolated from plants, animals, soil, water, and fecal matter. This genus contains 25 species and 2 subspecies. The taxonomy of this genus is evolving because of the improved taxonomic methodologies. Four of the 25 species (previously called *Pantoea intestinalis*, *Pantoea calida*, *Pantoea theicola*, and *Pantoea gaviniae*) were shifted to the genus *Mixta*, and three of them (formerly called *Pantoea terrea*, *Pantoea punctata*, and *Pantoea citrea*) were shifted to the genus *Tatumella*.<sup>6</sup> *Pantoea agglomerans* (*P. agglomerans*; previously called *Enterobacter agglomerans*, and *Erwinia herbicola*) is the representative member of the genus *Pantoea*.<sup>7,8</sup> These microorganisms are

facultative anaerobic and have an inherited capability to remain viable, grow well, and contaminate many inanimate objects at room temperature. Thus, they are among the most widespread bacteria in the environment and are the most frequent species of the genus *Pantoea* associated with human infections.<sup>9</sup>

Although *P. agglomerans* bacteria are mainly plant pathogens, they can cause human infections typically associated with thorn prick injuries or contaminated parenteral fluids. Generally, the infections are localized in healthy people with intact immune systems, while systemic severe infections are frequently reported in newborns, children, patients with comorbidities, or immunocompromised hosts.<sup>10,11</sup> *P. agglomerans* can cause wound infection in humans through skin piercing or laceration by any plant materials, such as thorns and wooden splinters that inoculate blood with the microorganisms, mostly during gardening, performing agricultural occupations, or children playing.<sup>12,13</sup> Another major reason for clinical *P. agglomerans* infections in humans is the exposure of hospitalized people (both adults and pediatrics) to fluids (such as blood products, propofol medications, intravenous fluids, and total parenteral nutrition) or medical equipment (such as IV catheters) contaminated with the organisms.<sup>10,13-15</sup> In Italy, an outbreak of *P. agglomerans*-induced sepsis was reported in a teaching hospital due to contamination of cotton pledgets by the hands of healthcare providers, or cross-contamination of the hospital environment *via* flowers gifted for patients that are left close to windows or in the corridors.<sup>16</sup> Furthermore, *P. agglomerans* is associated with an endotoxin-induced febrile response called “cotton fever” due to the accidental introduction of contaminated cotton to the bloodstream, especially if this cotton was used previously to filter substances before the intravenous injection.<sup>17</sup>

Fortunately, these bacteria are generally susceptible to most antibiotics. Thus, the proper antibiotic treatment leads to complete recovery of *P. agglomerans* infections in most cases.<sup>18,19</sup> However, with the emergence of antibiotic resistance problems, the bacteria contaminating CPs can be challenging to eradicate and represent a potential source of HAIs.<sup>20</sup>

Data on *Pantoea* species in Saudi Arabia, including its colonization, infections, and transmission, are scarce.<sup>21-24</sup> Concerns regarding the potential role of CPs in the transmission of HAIs are increased due to their cross-contamination in the areas of healthy people, then act as vehicles for bacterial transmission to the hospital, especially the most sensitive patient zones such as intensive care, isolation, and burn units. A better understanding of the possible modes of *P. agglomerans* transmission is required in Saudi Arabia to help health policymakers in its optimal prevention and control strategies. As far as we know, this is the first study in Saudi Arabia to check the potential role of medical students' CPs in *P. agglomerans* transmission to hospitalized patients and assess their antimicrobial susceptibility profile.

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## Materials and Methods

### Study design, and samples collection

The sample size was calculated using an online (Raosoft) sample size calculator (<http://www.raosoft.com/samplesize.html>) with a margin of error of 5%, response distribution of 50%, and confidence level of 95% for the total

700 students at the College of Medicine, Jouf University, Saudi Arabia, during the academic year of 2022/2023. After obtaining bioethical approval from the local committee of bioethics, Jouf University, Saudi Arabia, a cross-sectional study was performed to randomly swab 250 CPs of volunteering students during the intervals between the lectures after getting their permission and consent. Medical students who used CPs for longer than 2 months and consented to participate in the study meet the inclusion criteria. The randomly recruited participants were sequentially included in the study till the calculated sample size was achieved. Students who cleaned their CPs after learning about the study as well as medical students who did not use or own CPs at the time of the study were excluded.

Sterile powder-free gloves were used during the collection of samples to avoid cross-contamination. Sterile cotton-tipped swabs with amies transport media were used (GlobalRoll®, Hangzhou, China). The sterile swabs moistened with the transport media were firmly rolled over all exposed outer surfaces of the CPs. During swabbing, we focused on the areas that are most commonly in contact with ears, mouth, and tips of fingers, such as the microphone area, receiver, home button, and touch screen. The swabs were labeled carefully and transported in an icebox to the Microbiology and Immunology Laboratory at the College of Medicine, Jouf University for further processing.

### Bacterial isolation

The samples were processed using aseptic techniques to avoid contamination. The swabs were put in tubes containing 5 mL of double-strength brain-heart infusion broth (Oxoid, Hampshire, UK) and incubated aerobically for 24 hours at 37°C. The swabs were inoculated on blood and MacConkey agar plates (Oxoid, Hampshire, UK) and incubated aerobically for 24-48 hours at 37°C. To obtain a pure single colony of each bacterial isolate, the isolates were subcultured on blood and MacConkey agar plates and incubated aerobically at 37°C for 24 hours.

### Bacterial identification

The pure colonies of the isolates were identified by standard microbiological methods including Gram stain, size, color, and shape. Confirmation of isolates was done by the automated Vitek-2 compact system and GN-ID cards (BioMérieux, Marcy l'Etoile, France). The automated systems are widely used in clinical laboratories due to their numerous advantages, including accuracy, ease of use, rapidity, and reproducibility.<sup>25</sup>

### Antimicrobial susceptibility testing of the isolates

The antimicrobial susceptibility testing of the isolates was done by using the Epsilon tests (E-tests) (BioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions for rapid and accurate reporting.

### Quality control

The negative controls of the study during bacterial culture were non-inoculated blood and MacConkey agar plates

incubated aerobically for 48 hours at 37°C. Triplicate quality control testing was performed by using *P. agglomerans* ATCC 27155 strains (American Type Culture Collection, Manassas, VA, USA) as positive controls. Triplicate testing was done for each isolate.

## Data analysis

All the antibiotic susceptibility data were interpreted according to the Clinical and Laboratory Standards Institute.<sup>26</sup> The sensitivity, intermediate susceptibility, and resistance breakpoints are shown in Table 1.

## Results

The prevalence of *P. agglomerans* contamination of CPs of students at the College of Medicine, Jouf University, Saudi Arabia, was screened in 250 samples that were collected during the intervals between the lectures, after getting their permissions. Samples were processed and cultured on appropriate media under suitable incubation conditions. The bacterial isolates that were mucoid on MacConkey and yellowish mucoid on blood agar plates were suspected, stained by Gram stain, and examined microscopically. The Gram-negative bacilli were examined by the Vitek-2 compact system. A total of 51 isolates (20.40%; 51/250) were confirmed by the Vitek-2 compact system as *P. agglomerans*.

Regarding the antibiotic sensitivity of *P. agglomerans* isolates by E-tests, although all isolates were amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin/tazobactam, and sulfamethoxazole/trimethoprim sensitive, many isolates were intermediate/resistant to ampicillin, aztreonam, cefazolin, cefotaxime, cefuroxime, and ertapenem antibiotics with variable degrees as shown in Table 1.

## Discussion

Data on *Pantoea* species, including its colonization, infections, and transmission, are scarce in Saudi Arabia; therefore, the role of *P. agglomerans* in healthcare- and community-associated infections is underestimated. CPs can be easily contaminated in the areas of healthy people and then act as vehicles for bacterial transmission to healthcare settings. Considering that *P. agglomerans* occurs profusely in plants and animal products and the agricultural nature of Aljouf province in Saudi Arabia, this study is the first in Saudi Arabia to check the potential role of medical students' CPs in the transmission of *P. agglomerans* to hospitalized patients and to check the antibiotic susceptibility of the isolates to the most regularly used antibiotics.

During the study, a total of 250 CPs were swabbed. 51 isolates (20.40%; 51/250) were confirmed by the Vitek-2 compact system as *P. agglomerans*. This frequency of *P. agglomerans* detection is relatively high compared to other studies. Globally, many studies from different countries aimed to examine the bacterial contamination of CPs, but *P. agglomerans* organisms were not among the isolates.<sup>20,27-33</sup> In Riyadh, the capital of Saudi Arabia with an urban nature, *Pantoea* species were not detected in one study aimed to examine the bacterial contamination of CPs of healthcare workers in three hospitals,<sup>34</sup> and another study aimed to examine the bacterial contamination of CPs of visitors of many shopping centers.<sup>35</sup> Similarly, *Pantoea* species were not among the bacterial isolates detected in two older studies in AlKhubar and Taif cities of Saudi Arabia, where the CPs of 288 healthcare workers in King Fahd University Hospital and the CPs of 101 students and workers at Taif University were tested, respectively.<sup>36,37</sup> Furthermore, recent research in Jazan City, Saudi Arabia, did not detect *Pantoea* species among the bacterial isolates contaminating the CPs of healthcare workers.<sup>38</sup>

**Table 1.** Results of antimicrobial susceptibility testing of *Pantoea agglomerans* isolates by Epsilon tests.

Antibiotics	MIC (µg/mL) breakpoints*			Number of <i>Pantoea agglomerans</i> isolates; Total n=51 (100%)		
	Resistant	Intermediate	Sensitive	Resistant, n (%)	Intermediate, n (%)	Sensitive, n (%)
Amikacin	≥64	32	≤16	0 (0.00)	0 (0.00)	51 (100.00)
Ampicillin	≥32	16	≤8	1 (1.95)	1 (1.95)	49 (96.10)
Aztreonam	≥16	8	≤4	7 (13.75)	1 (1.95)	43 (84.30)
Cefazolin	≥8	4	≤2	0 (0.00)	8 (15.70)	43 (84.30)
Cefotaxime	≥4	2	≤1	3 (5.90)	3 (5.90)	45 (88.20)
Ceftazidime	≥16	8	≤4	0 (0.00)	0 (0.00)	51 (100.00)
Cefuroxime	≥32	16	≤8	4 (7.85)	1 (1.95)	46 (90.20)
Ciprofloxacin	≥1	0.5	≤0.25	0 (0.00)	0 (0.00)	51 (100.00)
Ertapenem	≥2	1	≤0.5	1 (1.95)	1 (1.95)	49 (96.10)
Gentamicin	≥16	8	≤4	0 (0.00)	0 (0.00)	51 (100.00)
Imipenem	≥4	2	≤1	0 (0.00)	0 (0.00)	51 (100.00)
Piperacillin/tazobactam	≥128/4	32/4-64/4	≤16/4	0 (0.00)	0 (0.00)	51 (100.00)
Sulfamethoxazole/trimethoprim	≥76/4	-	≤38/2	0 (0.00)	0 (0.00)	51 (100.00)

MIC, minimum inhibitory concentration; \*the breakpoints are defined by the Clinical and Laboratory Standards Institute.<sup>26</sup> The control *Pantoea agglomerans* strain (ATCC 27155) was sensitive to the tested antibiotics.

In Jeddah, a large city of urban nature in Saudi Arabia, *Pantoea* species were isolated but at a very low level (only one isolate, representing 0.95% of the CP contamination rate) in a study aimed to check bacterial contamination of 105 medical students' CPs at King Abdulaziz University.<sup>22</sup> Similarly, a recent study carried out in Riyadh and aimed at assessing the burden of bacterial contamination on 130 healthcare workers' CPs, identified only one *P. agglomerans* strain (representing 0.78% of the CP contamination rate) from a CP of one healthcare worker working in a medical ward at King Saud University Medical City in a study.<sup>24</sup> In the Nicosia province of Northern Cyprus, which has a large student community, a very low prevalence (3.0%) of *Pantoea* species isolation was detected in a study aimed at assessing the bacterial and fungal contamination loads of 233 students' CPs at the Faculty of Dentistry, Near East University.<sup>4</sup>

The relatively high prevalence *P. agglomerans* that was detected in the current study could be attributed to the agricultural nature of the Aljouf community and the decreased awareness of the tested group regarding *P. agglomerans* colonization, infections, transmission, prevention, and control. Due to the possibility that CP owners' hands frequently have higher rates of microbial contamination than everyone's CP, the presence of such potentially dangerous bacteria on CPs may be a sign of a larger problem. Therefore, it is strongly advised that awareness regarding *P. agglomerans* be raised in geographic areas with an agricultural tradition.

The greatest *P. agglomerans* healthcare-associated epidemic was reported in the USA: 152 septicemia cases were detected, with a 13.4% mortality rate due to contamination of intravenous fluid bottles' screwcaps by the organism.<sup>14</sup> A similar outbreak with a lower mortality rate (6.3%) was reported in Greece among children and infants due to contamination of intravenous fluid bottles' screwcaps by these bacteria.<sup>10</sup>

In Malaysia, contamination of parenteral nutrition solutions by *Pantoea* species resulted in a septicemia outbreak with a very high mortality rate (87.5%) among ICU neonates.<sup>15</sup> In Brazil, healthcare-associated outbreaks of *P. agglomerans* bloodstream infections were reported due to contamination of intravenous rehydration transference tube,<sup>39</sup> and an anticoagulant solution prepared in the hospital's pharmacy.<sup>40</sup>

The environmental nature of *P. agglomerans* microorganisms enables them to contaminate and survive on a variety of items found in healthcare settings including flowers, fruit brought by families, cotton swabs, cotton-based gauzes, and medical devices, or even the organisms may colonize a hospital unit.<sup>16</sup> Contaminated medical devices in healthcare settings by *P. agglomerans* resulted in many fatal complications, such as catheter-related bacteremia,<sup>12</sup> endocarditis in a mitral valve leaflet prolapse patient,<sup>41</sup> septicemia following blood transfusion,<sup>42</sup> pneumonia after heart-lung transplantation,<sup>43</sup> a ventilator-associated pneumonia,<sup>44</sup> catheter-associated septicemia,<sup>45</sup> and peritoneal dialysis catheter-associated peritonitis.<sup>46</sup>

Surprisingly, *P. agglomerans* can cause serious infections not only in immunocompromised patients (as mentioned above), but also in immunocompetent people; for example, pneumonia in a healthy British carpenter,<sup>47</sup> pneumonia in a healthy Spanish man,<sup>48</sup> septicemia in an immunocompetent Indian farmer.<sup>49</sup> Three cases of *P. agglomerans* bacteremia were reported in immunocompetent children.<sup>50-52</sup> The first case was after rotavirus gastroenteritis, and the authors postu-

lated that the *P. agglomerans* bacteria were translocated to the blood across the gut mucosa because of the gastroenteritis.<sup>50</sup> In the second case, the authors postulated the *P. agglomerans* bacteremia was induced after a urinary tract infection episode in a child with posterior urethral valve as a comorbidity.<sup>51</sup> In the third case, spontaneous *P. agglomerans* bacteremia was reported in an immunocompetent child without preceding infections and comorbidities.<sup>52</sup>

Recently, *P. agglomerans* bacteria were detected in a university teaching hospital in Zaria, Nigeria, as an uncommon cause of community-acquired bacterial infection in neonates. The authors reported eight cases of neonatal sepsis acquired from the community (though the source was not determined), with non-specific clinical presentation and sensitivity to most tested antibiotics, including gentamicin and ciprofloxacin.<sup>19</sup>

The above-mentioned data confirm the need to consider *P. agglomerans* as a potential cause of hospital-acquired and community-acquired infections with atypical clinical presentations, especially among neonates, immunocompromised people, or if the infection was preceded by injury with plant material. Finding the source of the pathogen might be difficult when investigating outbreaks in healthcare facilities. One theory is that the environment around the patients, such as curtains, mattresses, and linens, which have organisms with susceptibility patterns like the outbreak strain, might be the origin of such outbreak.<sup>53</sup> According to this viewpoint, the potentially contaminated CP with *P. agglomerans* should be considered during outbreaks with unusual clinical manifestations.

The relatively high contamination of CPs by *P. agglomerans* detected in this study is alarming because they can act as vehicles for bacterial transmission and cross-contamination. To limit the potential transmission of microorganisms through CPs, proper infection control practices should be implemented, such as hand hygiene and CP disinfection with 70% alcohol.<sup>4,22</sup> CPs' cleaning methods are of vital importance because alcohol-based products are not effective on physically non-clean surfaces.<sup>54</sup> Additionally, it is critical that hospitals have visual reminders, such as posters, regarding CP limitations, particularly in the departments housing immunocompromised patients.<sup>22</sup>

Given the ubiquitous usage of CPs, especially in medical teaching and healthcare settings, it is advised that hygiene rules, guidelines, and restrictions on their use be developed and implemented. As a result, this modest intervention could significantly lower the danger of CPs acting as possible vectors for potentially pathogenic microorganisms in medical teaching, healthcare, and community settings.<sup>55</sup>

The antibiotic susceptibility profiles of *P. agglomerans* isolates detected in the conducted study are another alarm because the organisms expressed variable degrees of reduced sensitivity to ampicillin, aztreonam, cefazolin, cefotaxime, cefuroxime, and ertapenem, as shown in Table 1. Fortunately, all *P. agglomerans* isolates were sensitive to amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin/tazobactam, and sulfamethoxazole/trimethoprim in the current study. These findings agree with the results of research at Near East University, Nicosia province, Cyprus, in which the detected *Pantoea* species were sensitive to all antibiotics tested, including amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin/tazobactam, sulfameth-oxazole/trimethoprim, and aztreonam.<sup>4</sup>

Unfortunately, the research team at King Abdulaziz University, Jeddah, Saudi Arabia, did not specify the member of

*Pantoea* species detected in their research, and they did not test its antimicrobial susceptibility.<sup>22</sup> Furthermore, the researchers at King Saud University Medical City, Riyadh, Saudi Arabia, did not test the antimicrobial sensitivity of the detected *P. agglomerans* isolate in their study.<sup>24</sup>

Generally, *P. agglomerans* bacteria are sensitive to most antibiotics.<sup>18,19</sup> However, the change in the susceptibility/resistance patterns of *P. agglomerans* against the antibiotics tested in the conducted study can be explained by the irrational use of antimicrobials in animal husbandry and agriculture that represent a selection pressure on the organism. The health policymakers should optimize the use of antimicrobials in agriculture, and animal husbandry. Moreover, they should think of new strategies to limit the spread of antimicrobial-resistant organisms such as vaccination.<sup>56</sup> Vaccination strategies could protect both humans and animals from antimicrobial resistance by reducing the infection risk and antibiotic prescription, thus limiting the emergence of antibiotic-resistant bacterial strains.<sup>57</sup>

With advances in science and technology, reducing the bioburden on CPs could be easier through implementing novel interventions to control bacterial contaminants. Limiting the number of CP's touchscreen edges is recommended.<sup>58</sup> Due to their antibacterial qualities, metallic nanoparticles can be utilized to enhance metal complexes in a range of applications, including the biological field.<sup>59</sup> Modern techniques can be utilized to lessen the bacterial contamination of CPs, but they still have significant limits. Examples include antibacterial wipes, antibacterial screens, and UV-light-based cabinets. The antibacterial wipes are instant, cost-effective, easy, and ready-to-use decontamination methods, but their disadvantages include the short-term antibacterial activity, which can cause scratches or even damage CPs' screens due to their abrasive nature. Although antibacterial screens (coatings) have many advantages including long-term antibacterial activity, broad-spectrum bactericidal activity, reduction in the development of antimicrobial-resistant bacteria, high biocompatibility (with minimal health risks), and reduced glare, their use is limited by the leaching of metal ions from antibacterial screens, silver toxicity at high concentrations, their higher cost than antibacterial wipes, and inability to be applied to the back and sides of CPs. UV-light-based cabinets are the most effective, as UV light can easily kill a broad range of bacteria within a few minutes with a longer lifetime and can be applied to the front, back, and sides of CPs. On the other hand, they are expensive with low biocompatibility (as UV exposure may cause health risks) and may fade CPs' screen colors.<sup>60</sup> Therefore, mobile manufacturers must offer in-depth instructions on phone cleaning and disinfection either inside the phone box or through their exclusive websites and applications to ensure an effective decontamination process without damaging the CPs.

The clinical significance of isolating *P. agglomerans* bacteria from medical students' CPs in terms of causing HAIs is dependent on many factors, including bacterial virulence, host susceptibility, and compliance with proper infection control measures, particularly hand hygiene. A limitation of our study was that we did not assess the survival time of *P. agglomerans* on the surface of CPs as a risk of their potential transmission to the hospitalized patients through CPs. However, the strength of the conducted study is that it increases the awareness and knowledge of medical students concerning *P. agglomerans* colonization, infections, transmission, prevention,

control, and the novel interventions for bacterial contamination control through the current advances in materials sciences and technology of CPs' antimicrobial surfaces.

## Conclusions

Considering that *P. agglomerans* occurs profusely in plants and animal products, and the agricultural nature of the locality, the results of the study could be valuable for health policymakers in implementing optimal infection prevention and control strategies. The study provides an alarm regarding the potential role of CPs, if they are not used cautiously, by acting as vehicles transmitting *P. agglomerans* to hospitalized patients, especially if immunocompromised, and the fatal complications that can be induced. The potentially contaminated CPs with *P. agglomerans* should be considered during outbreaks with unusual clinical manifestations. Developing CPs' antimicrobial surfaces, availability of posters about CPs restrictions in healthcare settings, and increasing the awareness of medical students regarding the transmission of *P. agglomerans* bacteria from healthy people's colonized zones to susceptible patients by involving them in medical training and educational programs concerning patient safety, infection prevention, and control during the preclinical phase of their study are of vital importance.

Further studies regarding *P. agglomerans* prevalence are required worldwide and in many Saudi regions, especially those of agricultural nature, with comparison of these results to evaluate the actual burden of these bacteria in the community and healthcare settings and try to limit their spread.

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