

## Enteral Nutrition Contamination as a Risk Factor for Ventilator-Associated Pneumonia in ICU Patients: A Study from Northeast Iran

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### ABSTRACT

**Introduction:** Ventilator-associated pneumonia (VAP) is a critical complication in hospitalized patients, particularly in intensive care units (ICUs). Evidence indicates an association between enteral nutrition (EN) contamination and nosocomial pneumonia, primarily through microbial colonization and aspiration. Accurate data on causative agents are vital for effective infection control. This 10-month study at Ghaem Hospital, Mashhad, Iran, assessed the incidence of VAP in ICU patients and evaluated the microbiological contamination levels of enteral nutrition and its association with VAP development. **Methods:** This prospective cohort study in an ICU evaluated 51 patients receiving EN via nasogastric tubes. Microbial contamination was assessed at four stages (preparation, ICU delivery, administration, and post-administration residual) (threshold  $\geq 10^3$  CFU/mL). Univariate and multivariable logistic regression analyses were employed to determine the independent association between enteral contamination and the development of VAP. **Results:** Out of 51 patients, VAP incidence was 60.8% (n=31). Significant contamination ( $\geq 10^3$  CFU/mL), dominated by Gram-negative bacilli, occurred predominantly at the bedside (Stages 3 and 4). Significant enteral contamination ( $\geq 10^3$  CFU/mL) was universally identified in all patients who developed VAP, underscoring its critical role as a primary reservoir and a key factor in the development of nosocomial pneumonia in this cohort. **Conclusion:** Contamination of enteral feeding solutions frequently exceeds safe microbiological thresholds and serves as a significant independent risk factor for VAP in ICU patients. Our findings underscore the critical importance of implementing rigorous aseptic protocols during the preparation and administration of enteral nutrition at the bedside. Adopting these measures is essential to mitigate infection risks and improve clinical outcomes in critically ill patients.

### INTRODUCTION

Ventilator-associated pneumonia (VAP) is a leading healthcare-associated infection in intensive care units (ICUs), with a reported incidence rate of 5% to 15%. Pneumonia in the ICU setting carries substantial mortality, often reaching as high as 50% [1], and is a critical acute complication affecting hospitalized patients [2, 3].

As the use of enteral nutrition (EN) continues to expand rapidly across clinical settings, healthcare personnel must be thoroughly educated on its associated complications, particularly microbial contamination risks and the potential for micro-aspiration, both of which are critical precursors to nosocomial infections [4]. EN is the preferred method of nutritional support for critically ill

patients who cannot maintain oral intake. However, the clinical benefits of EN are frequently offset by significant complications, most notably the risk of microbial contamination. Such contamination can lead to serious infectious outcomes, particularly nosocomial pneumonia, which remains a primary concern in intensive care settings [5, 6].

VAP is considered one of the most frequent types of hospital-acquired infections according to the World Health Organization (WHO) reports [7]. It is considered a threat to global health, especially among the elderly, and has a significant impact on patient mortality [8]. The high burden of VAP places severe pressure on healthcare systems, necessitating the widespread use of broad-

spectrum antimicrobials, including carbapenems, third- and fourth-generation cephalosporins, and fluoroquinolones. Such intensive therapeutic interventions significantly escalate the risk of emerging multidrug-resistant (MDR) pathogens [9].

The prevalence of Gram-negative bacteria in VAP cases is a major concern globally. Studies conducted in nearby countries, such as Georgia, as well as reports from Iranian ICUs, have consistently demonstrated a high burden of multidrug-resistant Gram-negative pathogens. These collective findings highlight a critical regional need to strengthen infection control practices [10, 11].

Most bacterial ventilator-associated or hospital-acquired pneumonia cases result from the aspiration of oropharyngeal or upper gastrointestinal secretions colonized by pathogenic organisms, a mechanism that aligns with the diagnostic framework established in the Centers for Disease Control and Prevention (CDC) guidelines [12, 13].

Enteral feeding via nasogastric tubes is well-recognized as a significant risk factor for the development of nosocomial pneumonia [14, 15]. This association is primarily linked to the potential for nasal colonization and subsequent aspiration, emphasizing the need for rigorous monitoring of EN delivery systems.

Previous studies have established a significant correlation between the microbial contamination of EN and the development of VAP [16]. Despite this established link, there is a scarcity of comprehensive data detailing contamination patterns across the specific stages of feed preparation and administration. In Iran, recent systematic reviews and local epidemiological studies indicate a high burden of ICU-acquired respiratory infections, with VAP incidence rates frequently reported between 20% and 30% in tertiary care settings [17]. Despite this, systematic evaluations of EN-related contamination throughout the delivery process remain limited [18].

This lack of regional evidence, combined with variations in local infection control practices, underscores the urgent need for a targeted investigation into the contamination profiles of enteral feeds in Iranian ICU settings.

The present study was designed to address this gap by evaluating enteral feeding contamination across four stages and examining its association with VAP in ICU patients at Ghaem Hospital, Mashhad.

## METHODS

**Study design and setting.** This was a prospective observational cohort study conducted over 10 months (Sep 2018 to Jun 2019) at the Intensive Care Unit of Ghaem Hospital, Mashhad, Iran. Patients were followed from the initiation of enteral feeding until hospital discharge, death, or a confirmed diagnosis of VAP.

## Participants and inclusion/exclusion criteria.

**Inclusion Criteria:** Patients aged  $\geq 18$  years, admitted to the ICU, who initiated enteral feeding via a nasogastric (NG) tube and received this mode of nutrition for at least 48 hours.

**Exclusion Criteria:** Patients admitted with a diagnosis of pneumonia, those whose NG feeding tube was discontinued before 48 hours, or patients with underlying severe immunodeficiency (*e.g.*, active acquired immunodeficiency syndrome (AIDS) or recent organ transplantation).

**Data collection and risk factors.** Demographic, clinical, and laboratory data, alongside potential risk factors for VAP, were prospectively recorded for each patient using a structured questionnaire based on the CDC/NHSN criteria [12].

The questionnaire captured data on underlying illnesses (*e.g.*, diabetes, immunosuppression), device-related factors (*e.g.*, endotracheal intubation, mechanical ventilation, NG tube placement, enteral feeding), and medication use (*e.g.*, antibiotics, antacid drugs). The diagnostic criteria used to define VAP in this study are detailed below.

**Definition of nosocomial pneumonia (HAP and VAP).** The diagnosis of VAP was established based on the CDC/NHSN criteria [19]. For patients who were not receiving mechanical ventilation, Hospital-Acquired Pneumonia (HAP) criteria were applied [19].

However, for patients who required mechanical ventilation during their ICU stay, the diagnosis was confirmed using VAP criteria [20]. Both diagnoses required the presence of three concurrent elements:

**Clinical findings:** At least two of the following: fever (temperature  $> 38.0$  °C), leukocytosis (WBC  $> 12,000/\text{mm}^3$ ) or leukopenia (WBC  $< 4,000/\text{mm}^3$ ), and purulent respiratory secretions.

**Physical examination:** New onset of cough, dyspnea, or deterioration of gas exchange.

**Microbiological confirmation:** Respiratory infections were confirmed by quantitative or semi-quantitative cultures of endotracheal aspirate (ETA) or sputum. In accordance with CDC/NHSN guidelines, a growth of  $\geq 10^5$  CFU/mL for ETA samples was considered significant for identifying the causative agent. For pleural fluid samples, any growth was considered clinically relevant.

For patients on mechanical ventilation, the diagnosis of VAP was established using the CDC/NHSN clinical and laboratory criteria (PNEU 1/2) [20]. Both diagnoses required the presence of three concurrent elements:

**Radiologic evidence:** New or progressive and persistent infiltrate, consolidation, or cavitation on chest X-ray.

**Systemic signs:** At least one of the following: fever ( $> 38$  °C) with no other recognized cause, or abnormal white blood cell count  $\geq 12,000$  or  $\leq 4,000$  cells/ $\text{mm}^3$ .

Microbiological/clinical evidence: New onset of purulent sputum, or positive quantitative culture from endotracheal aspirate ( $\geq 10^5$  CFU/mL) obtained  $\geq 48$  hours after endotracheal intubation.

#### Contamination assessment and sampling protocol.

For the assessment of the enteral feeding solution, sampling was conducted throughout the patient's feeding cycle in four different stages:

**Stage 1:** The first sample was taken from the central feeding solution immediately after preparation in the hospital kitchen.

**Stage 2:** The second sample was collected after transportation and delivery to the ICU.

**Stage 3:** The third sample was obtained during administration to the patient through an NG tube.

**Stage 4:** The final sample was collected from the gastric residual volume aspirated via the feeding tube two hours after the completion of the bolus administration.

All samples were handled using aseptic techniques, including sterile gloves, masks, and disinfected surfaces. Sample collection was performed by a dedicated team of trained ICU nurses who were strictly briefed on the study's aseptic protocol. To ensure consistency, all personnel followed a standardized procedure for each of the four sampling stages throughout the study period.

Environmental controls were conducted to rule out extrinsic contamination. Throughout the study, all random swabs from air, working surfaces, and personnel's hands yielded no growth of pathogens, confirming the integrity of the aseptic sampling process.

**Feeding regimen and protocol.** All patients received a blenderized diet prepared daily in the hospital's central kitchen. The feeding protocol was as follows:

- Administration method: The formula was administered every 4 hours using the intermittent bolus technique via 60 mL sterile syringes.
- Timing: To ensure freshness and minimize microbial growth, the formula was delivered to the ward in sealed containers and administered immediately (within 30 minutes of arrival).
- Volume and rate: Each bolus consisted of approximately 250 mL, injected slowly over 15 minutes to prevent gastric distress.
- Equipment hygiene: Syringes and NG tubes were flushed with sterile water before and after each feeding, and syringes were replaced every 24 hours, in accordance with the hospital's infection control protocol. Between feedings, syringes were stored in a clean, dry container to prevent environmental contamination.
- Patient positioning: The head of the bed was elevated to 30–45 degrees during and for 1 hour after administration to reduce the risk of aspiration.

The time from sampling to inoculation on culture media did not exceed 30 minutes to minimize bacterial overgrowth and ensure accurate CFU counts.

Ten samples were collected for Stage 1 and Stage 2 on randomly selected days during the study period to assess baseline and transportation-related contamination of the bulk enteral solution.

Samples for Stages 3 and 4 were collected from the enteral feeding solutions of all patients who received enteral nutrition via NG tube during the study period.

Of the 61 enrolled patients, 51 completed the sampling protocol and were included in the final analysis. Consequently, samples for Stage 3 and Stage 4 were collected from the enteral feeding solutions of all patients who met the inclusion criteria and completed the tracking protocol. This design ensured that the longitudinal assessment of microbial contamination during administration and post-administration residual phases was conducted systematically across the analytical cohort.

**Microbiological procedures.** The samples underwent 10-fold serial dilutions ranging from  $10^{-1}$  to  $10^{-9}$  using sterile normal saline solution. A total of 0.2 mL from each diluted sample was plated in duplicate: 0.1 mL onto sheep blood agar and 0.1 mL onto MacConkey agar. The samples were spread evenly across the plate surface using a sterile spreader. Plates were then incubated at 35–37°C for 24–48 hours under aerobic conditions. For suspected fastidious organisms, a 5% CO<sub>2</sub>-enriched atmosphere was utilized to ensure optimal recovery of all potential pathogens.

The time from sampling to inoculation on culture media did not exceed 30 minutes to minimize bacterial overgrowth and ensure accurate CFU counts.

For definitive species identification of Gram-negative bacilli (such as *P. aeruginosa*, *Acinetobacter* spp., and *K. pneumoniae*), a comprehensive panel of biochemical tests was employed. In addition to the Oxidase, Triple Sugar Iron (TSI), Urease, and Motility tests, Citrate utilization and Indole production tests were performed. Furthermore, standardized microbiological identification galleries (multi-test systems) were utilized to confirm the species-level identification, ensuring the accuracy of the reported pathogens.

**Statistical analysis.** Data were analyzed using the Per-Protocol (PP) approach. Patients with incomplete sampling stages or missing clinical data due to early discharge, death, or transition to oral feeding were excluded from the final analysis to ensure the integrity of the microbial comparison across all four stages. Missing data were not imputed, and only complete cases were included in the statistical models.

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25.0. Descriptive statistics were used to summarize patient demographics and microbial characteristics.

The primary exposure was defined as significant enteral nutrition contamination, using a threshold of  $\geq 10^3$  CFU/mL in the feeding solution at either Stage 3 or Stage 4 [21]. For this study, a patient was classified as exposed if significant contamination was detected in at least one of the samples collected during their ICU stay. Each patient underwent the four-stage sampling protocol once to capture the microbial profile during a single feeding cycle.

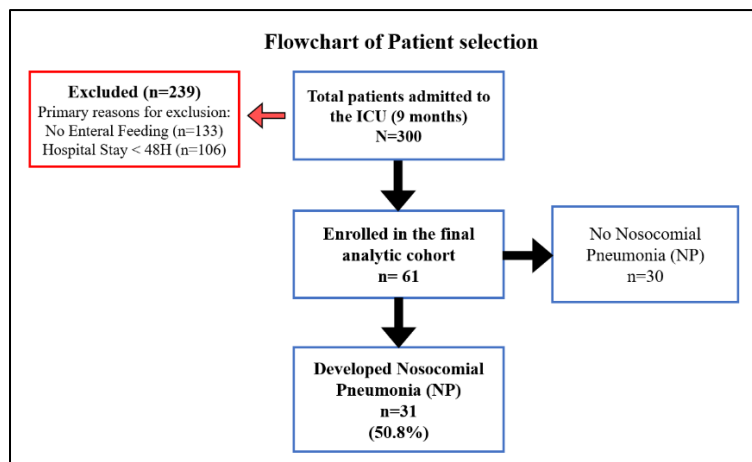
To determine the association between enteral contamination and the occurrence of VAP, a structured regression approach was employed. First, univariate logistic regression was performed, including variables such as age, sex, ICU length of stay, duration of NG tube placement, and the use of antibiotics during the ICU stay. Variables with a *P*-value < 0.20 in the univariate analysis, or those with known clinical significance, were entered into the multivariable logistic regression model.

The model's goodness-of-fit was assessed using the Hosmer-Lemeshow test, and multicollinearity was evaluated using the Variance Inflation Factor (VIF) to ensure the model's stability. This allowed for the calculation of the adjusted Odds Ratio (OR) and 95%

confidence intervals (CI), evaluating the independent role of contamination as a risk factor for the development of VAP. A *P*-value of less than 0.05 was considered statistically significant.

## RESULTS

**Patient enrollment and flow.** During the ten-month study period, a total of 300 patients were admitted to the ICU. After applying the inclusion and exclusion criteria, 61 patients were initially enrolled. Throughout the study, all random swabs from air, working surfaces, and personnel's hands yielded no growth of pathogens, confirming the integrity of the aseptic sampling process. However, 10 patients were excluded during the study: 6 patients were transitioned to oral feeding before the 48-hour mark, and 4 patients died before the completion of the sampling protocol. Consequently, 51 patients completed the study and were included in the final per-protocol analysis. Among this cohort, 31 patients (60.8%) developed VAP according to the CDC/NHSN criteria [19] (Figure 1).



**Fig. 1.** Flowchart of patient selection and outcomes in the ICU cohort

**Baseline characteristics and clinical outcomes.** The demographic and clinical characteristics of the analyzed cohort (n=51) are summarized in Table 1. Of the 51 patients, 21 (41.2%) were male. The majority of the cohort (92.2%, 47/51) required endotracheal intubation and mechanical ventilation. Regarding underlying conditions, diabetes mellitus was present in only 1 patient (1.96%). No cases of severe immunosuppression were

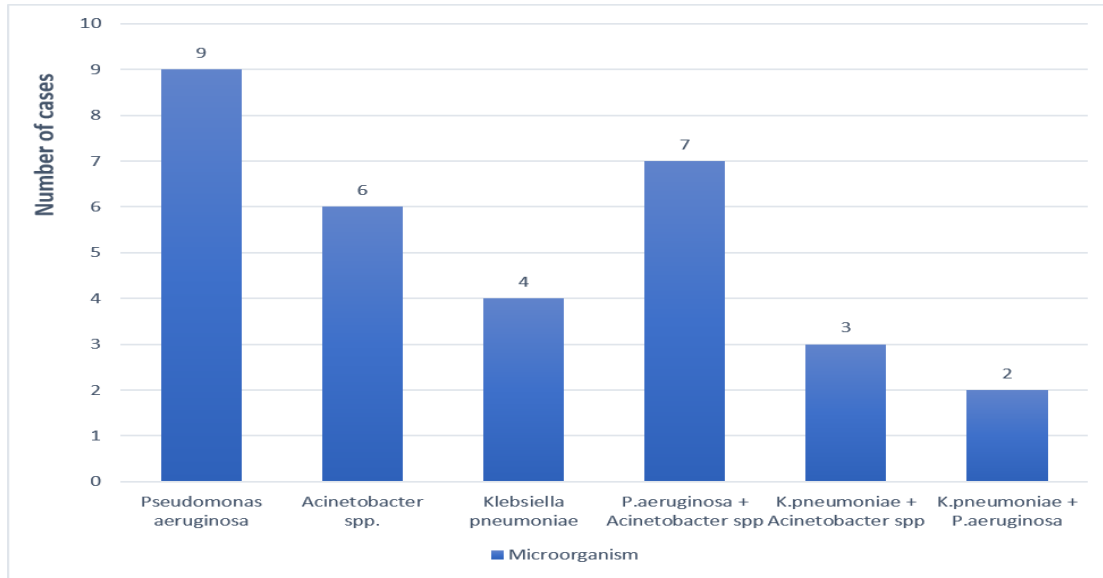
included. For the 31 patients who developed VAP, the ages ranged from 26 to 79 years. In this subgroup, 18 (58.1%) were female, and 13 (41.9%) were male. The overall mortality rate in the VAP group was 58.1% (18/31), with 10 females and 8 males dying during the ICU stay, while 41.9% (13/31) were successfully discharged.

**Table 1.** The demographic characteristics and clinical outcomes of the patients

Variable	Total Cohort (n=51)	VAP Group (n=31)
Age range	26 – 79	26 – 79
Gender (female)	30 (58.8%)	18 (58.1%)
Gender (male)	21 (41.2%)	13 (41.9%)
Clinical outcome (death)	--	18 (58.1%)
Clinical outcome (discharged)	--	13 (41.9%)
Comorbidities (diabetes mellitus)	1 (1.96%)	1 (3.2%)
Interventions (mechanical ventilation)	47 (92.2%)	29 (93.5%)

**Microbiological characteristics of VAP.** Among the 31 patients who developed ventilator-associated or hospital-acquired pneumonia, a total of 43 bacterial isolates were recovered from positive respiratory cultures. Monomicrobial infections were documented in 19 patients, while polymicrobial infections involving the co-isolation of two pathogens were identified in 12 patients. Specifically, polymicrobial cases consisted of *Pseudomonas aeruginosa* combined with *Acinetobacter*

spp. (n = 7), *Klebsiella pneumoniae* combined with *Acinetobacter* spp. (n = 3), and *Klebsiella pneumoniae* combined with *Pseudomonas aeruginosa* (n = 2). Among the monomicrobial infections, the recovered pathogens were *Pseudomonas aeruginosa* (n = 9), *Acinetobacter* spp. (n = 6), and *Klebsiella pneumoniae* (n = 4) (Figure 2). The main organisms identified across the four stages of sampling are presented in Table 2.



**Fig. 2.** Distribution of bacterial isolates recovered from ventilator-associated and hospital-acquired pneumonia cases (total of 43 isolates obtained from 31 patients, reflecting the presence of polymicrobial infections).

**Microbiological correlation between EN and respiratory cultures.** Gram-negative bacilli predominated in both enteral nutrition samples and respiratory cultures. Specifically, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were the leading pathogens in contaminated EN samples (Stages 3 and 4) as well as in VAP cases. Although systematic molecular concordance analysis was not performed, the high prevalence of the same species in both reservoirs supports an association between enteral contamination and VAP.

**Risks for VAP.** The clinical and demographic characteristics of the cohort, alongside their association

with the incidence of VAP, were summarized. Univariate analysis indicated that factors such as age, sex, and ICU length of stay did not differ significantly between patients with and without VAP ( $P > 0.05$ ). Notably, significant enteral contamination ( $\geq 10^3$  CFU/mL) was identified in all (100%) patients who developed VAP. This universal presence of significant microbial burden in the enteral nutrition of affected patients underscores its critical role as a primary reservoir and a key factor in the development of ventilator-associated pneumonia in this high-risk ICU population (Table 3).

**Table 2.** Distribution of selected bacterial isolates in enteral feeding samples across the four sampling stages

Stage	Number of samples	<i>Pseudomonas aeruginosa</i>		<i>Acinetobacter</i> spp.		<i>Klebsiella pneumoniae</i>	
		Negative	Positive	Negative	Positive	Negative	Positive
Stage 1 (Hospital kitchen)	10	10	0	10	0	10	0
Stage 2 (ICU delivery)	10	10	0	10	0	10	0
Stage 3 (During administration)	51	28	23	35	16	39	12
Stage 4 (Gastric residual aspirate)	51	28	23	35	16	39	12

**Table 3.** Logistic regression analysis of risk factors for ventilator-associated pneumonia (n=51)

Variable	Univariate P-value	Multivariate aOR (95% CI)	Multivariate P-value
Age	0.45	--	--
Sex (female vs. male)	0.62	--	--
Diabetes mellitus	0.12	1.2 (0.5 – 2.8)	0.54
Duration of NG tube placement	0.08	1.5 (0.8 – 3.1)	0.18
Enteral contamination ( $\geq 10^3$ CFU/mL)	< 0.01	4.8 (2.1 – 11.0)	< 0.01

Footnote 1: Dashes (--) indicate variables not retained in the final multivariable model.

Footnote 2: CI: confidence interval; aOR: adjusted odds ratio

## DISCUSSION

The gastro-pulmonary route is a well-recognized pathway in the pathogenesis of pneumonia, where the stomach acts as a reservoir for pathogens [22, 23]. The presence of an NG tube bypasses the natural protective barriers of the upper airway, facilitating chronic micro-aspiration of colonized gastric contents into the lungs [17].

A notable finding of our study was the complete absence of microbial growth in Stage 1 (preparation) and Stage 2 (initial delivery), contrasting sharply with the heavy contamination observed in Stages 3 and 4. This disparity indicates that the microbial burden does not originate from the hospital kitchen or the initial preparation phase. Instead, the high bacterial loads identified at the bedside point toward exogenous acquisition linked to bedside handling and possible prolonged hang times. Factors such as inadequate hand hygiene during administration, frequent disconnection of the feeding circuit, and the inherent risks of 'open systems' likely facilitate the introduction and rapid proliferation of environmental pathogens. Consequently, our findings demonstrate a strong clinical link between enteral nutrition contamination and the incidence of VAP in ICU patients, with significant contamination consistently observed in all patients who developed respiratory infections, highlighting its role as a critical reservoir for pathogen transmission.

The incidence of VAP in our analyzed cohort was 60.8% (31/51). While this rate is higher than ranges commonly reported for hospital-acquired or ventilator-associated pneumonia in broader ICU populations, this likely reflects the particularly high-risk nature of our cohort. Our inclusion criteria were limited to patients requiring prolonged enteral nutrition via nasogastric tubes for at least 48 hours, a population inherently more susceptible to micro-aspiration and colonization. This concentrated risk profile, combined with the open-system feeding method used in our setting, likely accounts for the higher observed incidence compared to general ICU surveillance data.

The presence of Gram-negative bacilli in EN solutions at levels exceeding the critical threshold ( $\geq 10^3$  CFU/mL) established as an unsafe limit by previous foundational studies [21] underscores the clinical severity of our findings. Our data suggest that contaminated EN serves as a substantial reservoir for bacterial colonization, which is

significantly associated with an elevated risk of VAP. This relationship is further validated by both univariate and multivariable logistic regression analyses, confirming EN contamination as a robust predictor of respiratory infection.

The significant microbial load observed in the enteral formulas (exceeding  $10^3$  CFU/mL) indicates a failure in bedside hygiene protocols rather than a baseline contamination during preparation. This high density of pathogens is clinically relevant, as it increases the probability of colonizing the gastric reservoir and subsequent micro-aspiration.

This microbiological evidence supports classifying these samples as significantly contaminated and reinforces the observed statistical association.

This is consistent with previous studies showing an increased incidence of nosocomial pneumonia associated with the colonization of opportunistic Gram-negative pathogens in enteral nutrition. While the present study did not systematically analyze the antimicrobial resistance patterns of the isolates, the types of bacteria identified, specifically *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are among the most common pathogens implicated in ICU-acquired infections globally [10, 24].

Consistent with previous research highlighting the role of enteral feeding contamination in ICU-acquired infections, our study demonstrates that this risk is primarily concentrated at the bedside administration phase. Unlike some studies that focus on metabolic complications or feeding intolerance, our findings specifically implicate the microbial integrity of the enteral formula as a critical determinant of respiratory outcomes. The high prevalence of pathogens in the enteral circuit, combined with the mechanism of micro-aspiration, underscores the need for more stringent infection control measures during the delivery of enteral nutrition.

Similarly, a multicenter study of 150 ICU patients in Iran reported that only 5.3% of the participating centers followed standardized feeding protocols, while 68.6% relied on handmade hospital formulas [25]. These factors, along with the limited use of post-pyloric feeding routes (3.3%), were linked to a higher incidence of complications and suboptimal nutritional outcomes, reinforcing the need for standardized enteral care to minimize risks.

Together, these findings highlight the importance of strict procedural adherence to minimize contamination risk.

Research has shown that inadequate handling of enteral feeding solutions, prolonged feeding tube use, and improper hygiene practices contribute to microbial colonization and subsequent pneumonia [21, 24].

A critical finding in our study was the species-level concordance observed between EN samples and respiratory cultures. In 22.6% (7/31) of the patients who developed VAP, the same pathogenic species (notably *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) were isolated from both the contaminated enteral formula at the bedside and the respiratory tract. This concordance provides circumstantial evidence supporting a possible gastro-pulmonary route of infection. While prior antibiotic therapy in other patients may have influenced the culture results, this subset of matching isolates reinforces the role of contaminated EN as a direct reservoir for respiratory pathogens.

The recovery of these organisms aligns closely with the major pathogens identified in the IDSA/ATS guidelines for HAP/VAP management [13], highlighting the clinical relevance of these pathogens in this patient population.

The pathogens identified in our study, such as *P. aeruginosa* and *A. baumannii*, are well known for their ability to form biofilms on the internal surfaces of NG tubes. These biofilms act as persistent microbial reservoirs that are highly resistant to standard flushing and antimicrobial agents. The presence of a mature biofilm in the NG tube not only facilitates the continuous contamination of the enteral formula as it passes through the circuit (consistent with our high CFU counts in Stage 4) but also increases the likelihood of retrograde colonization and micro-aspiration of these protected pathogens into the respiratory tract. This mechanism likely explains why infection control remains a major challenge despite routine handling protocols [25, 26].

Despite its clinical implications, this study has several limitations. First, the sample size was limited to a single center, and the use of an open feeding system may limit the generalizability of the findings to facilities using closed systems. Second, while we observed overlap at the species level, the lack of molecular strain-typing (e.g., pulsed-field gel electrophoresis (PFGE)) and antimicrobial susceptibility data prevents us from definitively confirming the genetic identity and resistance patterns of the isolates. Third, potential selection bias may exist as only patients meeting specific clinical criteria were included. Fourth, we did not control for all aspiration risk factors, such as patient positioning during feeding or gastric residual volumes, which could act as confounders. Additionally, unmeasured confounders such as hospital hygiene policies and antibiotic use patterns may have influenced the results. Finally, the single-time-point microbial sampling during feeding cycles means that a strict temporal relationship between contamination and the onset of VAP could not be established in all cases.

Future multi-center, prospective studies with molecular typing are needed to address these gaps.

Future research should prioritize the evaluation of standardized, evidence-based hygiene protocols, specifically comparing the efficacy of closed-system versus open-system enteral delivery in reducing bedside contamination. Additionally, prospective studies are needed to determine whether intensive staff training on aseptic handling techniques and the implementation of routine microbiological monitoring of enteral formulas can significantly lower the incidence of nosocomial pneumonia in high-risk ICU settings.

External evidence suggests that closed feeding systems significantly reduce the risk of exogenous contamination compared with open systems, as they minimize the number of times the circuit is breached and exposed to the bedside environment [27].

Future multicenter studies with expanded cohorts are essential to validate these findings across diverse clinical settings. Furthermore, integrating molecular techniques, such as the polymerase chain reaction (PCR), would be crucial for conducting strain-level concordance analysis. This would allow for definitive confirmation of the genetic identity between enteral pathogens and respiratory isolates, providing more robust evidence for the transmission pathway.

In conclusion, our study identifies a significant clinical association between microbial contamination of enteral feeding solutions and the development of VAP in the ICU. While this observational evidence points toward contaminated enteral nutrition as a potential independent risk factor, the definitive causal pathway requires further validation through molecular strain-typing. Based on these findings, we recommend the transition from open to closed-system delivery and the enforcement of strict hang-time limits (not exceeding 4 hours for open systems) to mitigate bedside proliferation. Furthermore, integrating standardized aseptic handling training for nursing staff and routine microbiological monitoring of formulas at the bedside are essential, actionable strategies to minimize exogenous contamination and enhance patient safety.

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#### CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest associated with this manuscript.

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#### AI DISCLOSURE

No artificial intelligence tools were used during manuscript preparation.

#### AUTHORS' CONTRIBUTIONS

BN: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. EHM: Investigation, Data curation, Validation, Writing – review & editing. KG: Supervision, Conceptualization, Methodology, Writing – review & editing, Project administration.

#### DATA AVAILABILITY

De-identified data may be available from the corresponding author upon reasonable request and with ethics approval, subject to hospital privacy policies.

#### ETHICS STATEMENT

The study protocol was conducted in accordance with the ethical principles of the Declaration of Helsinki and was formally approved by the Research Ethics Committee of Mashhad University of Medical Sciences (Approval Date: October 10, 2018; Ethical Code: IR.MUMS.MEDICAL.REC.1397.181; Project Code: 961737). Patient privacy and data confidentiality were strictly maintained; all personal identifiers were removed from the dataset, and data were stored in a secure, password-protected system. Considering the observational nature of the study and that sampling from the enteral circuit and respiratory tract was part of routine clinical monitoring or posed minimal risk, the requirement for written informed consent was waived by the Ethics Committee. Administrative permission for data collection was also obtained from the management and supervising clinical staff at Ghaem Hospital.

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