

## Risk of Colonization of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) and Vancomycin-Resistant Enterococci (VRE) in Patients admitted to Pediatric Intensive Care Unit of Ain Shams University Hospitals

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### ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) have been recognized as important nosocomial pathogens worldwide. This study was designed to determine the risk of colonization of MRSA and VRE in patients admitted to the pediatric intensive care unit (PICU) of Ain Shams University Hospitals and its association with predisposing factors.

All patients admitted to the PICU from October 2004 to March 2005 were assessed clinically and screened for MRSA and VRE using nasal, axillary and rectal swabs taken within 24 hours after admission and three days later. Risk factors analysis included age, length of hospital stay prior to PICU admission, antimicrobial treatment, application of ventilators and severity of critical illness as assessed by Physiological Stability Index (PSI) scoring system.

Of the 67 admissions, 17 were positive for MRSA and 3 were positive for VRE as detected through screening swabs. Of the 50 negative for MRSA on admission, 19(38%) were newly colonized and 11 of them were also vancomycin resistant (VRSA). Of the 64 initially negative to VRE, only one was newly colonized. Factors associated with MRSA acquisition were age younger than 3 years (RR= 3.2, 95% CI= 2-4.4), history of pre ICU use of anti-Gram positive antibiotics (RR= 4.1, 95% CI 95%=2.5-5.6) and application of ventilators during ICU stay (RR= 2.4, 95% CI = 1.5-3.8).

In conclusion, MRSA acquisition occurred frequently in PICU, thus control of MRSA would better be accomplished by screening for MRSA on admission to identify imported cases. Moreover, education concerning the importance of infection control measures as proper aseptic technique concerning mechanical ventilation and barrier protection from colonized patients as well as strict limitations of use of antimicrobials should be emphasized.

### INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) have emerged as prominent nosocomial pathogens worldwide (**Puzniak et al., 2002 and Gastmeier et al., 2003**). The increasing incidence of MRSA and VRE is problematic because of the limitations of effective treatment and eradication strategies leading to prolonged patient suffering, high mortality and increased hospital care costs (**Perl, 1999 and Punziak et al., 2001**). A more imminent threat concerning VRE is in the potential transfer of resistance genes to other species such as *Staphylococcus aureus* (**Perl, 1999, Cetinkaya, 2000 and Punziak et al., 2001**).

The reasons for the emergence of these organisms are multifactorial and can be attributed to antimicrobial pressures (**Punziak et al., 2001 and Graffunder and Venezia, 2002**), inconsistent application of infection control practices (**Cosseron-Zerbib et al., 1998, Neely and Maley, 2000 and Thompson, 2004**) and host factors such as severity of illness and length of hospital stay (**Campbell et al., 2003 Marshall et al., 2003, Tai et al., 2004 and Thompson, 2004**).

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Patients in intensive care units (ICUs) are at higher risk of acquiring nosocomial infections because of the severity of their underlying illnesses, extensive use of broad-spectrum antimicrobial agents, numerous invasive procedures, and extended hospital stays (**Punziak et al., 2001, Marshall et al., 2003 and Thompson, 2004**). In addition, these patients are often colonized with drug-resistant pathogens at the time of admission and become reservoirs for the horizontal transmission of nosocomial infections in hospital settings (**Punziak et al., 2001**).

To date, most national and international surveillances and prevention activities have been focused on adults despite the fact that pediatric patients are at greater risk of nosocomial infection. This may be because of their immature immune systems, prevalent device use and prevalent antimicrobial resistant organisms for which more costly broader spectrum antimicrobials are used for empiric therapy in the PICU (**Gina et al., 2004**).

The purpose of this study was to determine the risk of colonization of MRSA and VRE in patients admitted to the pediatric intensive care units (PICUs) of Ain Shams University Hospitals and its association with predisposing factors.

## **PATIENTS AND METHODS**

This was a 6 month prospective cohort study conducted on patients admitted to the 8-bed PICU of Ain Shams Pediatric Teaching Hospital during the period from October 2004 to March 2005.

Patients were assessed clinically with full history taking (age, sex admission to hospitals over the last 12 months, pre ICU hospital stay and antimicrobial use prior to ICU admission), thorough clinical examination and scoring the severity of critical illness using Physiological Stability Index (PSI), which is the *modified pediatric APACHE scoring system (APACHE=Acute Physiology and Chronic Health Evaluation)* for critical illness evaluation (**Pollack et al, 1987**). Prospective data collection occurred for all patients admitted to the PICU for more than 48 hours which included use of vascular catheters and use of mechanical ventilation.

All the children were screened for MRSA and VRE acquisition using nasal, axillary and rectal swabs. Swabs were taken from the patients within 24 hours of admission and three days later.

Swabs were inoculated directly on mannitol salt agar. Plates were incubated at 37°C for 48 hours. Individual colonies were streaked on sheep blood agar and incubated at 37°C overnight. Morphologically distinct colonies were tested for production of coagulase. All coagulase positive staphylococci were screened for methicillin resistance on Muller Hinton agar by disk diffusion according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (**NCCLS, 1997a**). MRSA strains susceptibility to vancomycin was tested also by disk diffusion method (**NCCLS, 1997a**). Resistance was confirmed by MIC testing using broth dilution method as recommended by **NCCLS, 2003**. MRSA was considered to be vancomycin resistant (VRSA) when the MIC of vancomycin was  $\geq 32\mu\text{g/ml}$  (**NCCLS, 2003**).

Rectal swabs were also directly inoculated onto bile esculin agar for detection of enterococci. Plates were incubated at 37°C for 48 hours. Esculin positive colonies (showing brown to black halo) were subjected to Gram staining and further biochemical testing for characterization of *Enterococci* (**Facklam et al., 1999**). The vancomycin susceptibility of *Enterococci* was determined by disk diffusion following the NCCLS methods (**NCCLS, 2001**). Resistance was confirmed by means of MIC testing with broth dilution method (**NCCLS, 1997 b**). Isolates for which the MIC of vancomycin was  $\geq 32\mu\text{g/ml}$  were classified as resistant.

Samples from patients who developed infection were collected according to the site of infection and examined in the same manner.

Initial comparisons between patients with MRSA positive and MRSA negative were done using the student's t-test for continuous variables and Pearson's Chi square test for categorical variables. The adjusted risk factors for MRSA were obtained using the logistic regression analysis. The dependent variable was the presence and absence of MRSA in all the patients. All variables

described previously were considered as possible candidates for the final model. Before accepting a final model, the interactions as well as confounding were evaluated. The calibration of the final model was assessed using the Hosmer and Lemeshow goodness-of-fit test, and its discrimination was assessed by the area under the receiver operator characteristic (ROC) curve.

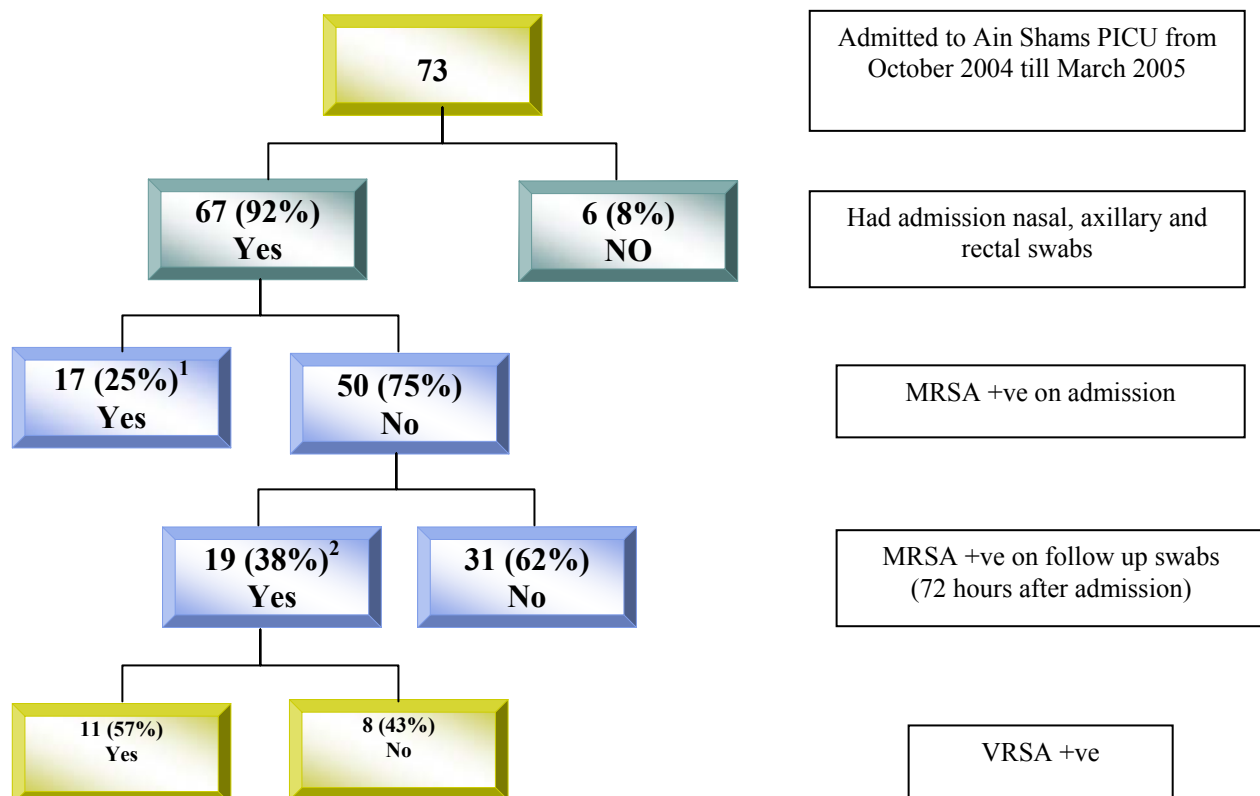
All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 10.0.

## RESULTS

During the study period, 73 patients were admitted to the PICU and stayed for more than 48 hours. Of these, 67 (92%) were screened using nasal, axillary and rectal swabs and were therefore eligible for study.

The mean age of this study group was 6.5±3.9 years. Their diagnosis in the PICU varied as 30% with bronchopneumonia, 9% with malnutrition complicated with bronchopneumonia, 18% with malignancy, 12% with severe gastroenteritis and dehydration, 7.5% with liver cell failure, 10% with heart failure complicating congenital heart diseases, 4.5% with renal failure, 6% with acute severe asthma and impending respiratory failure, and 3% with severe acute hematemesis. Seventeen patients (25%) were found to be colonized with MRSA and 3 patients (4%) were found to be colonized with VRE on admission [Figure 1&2].

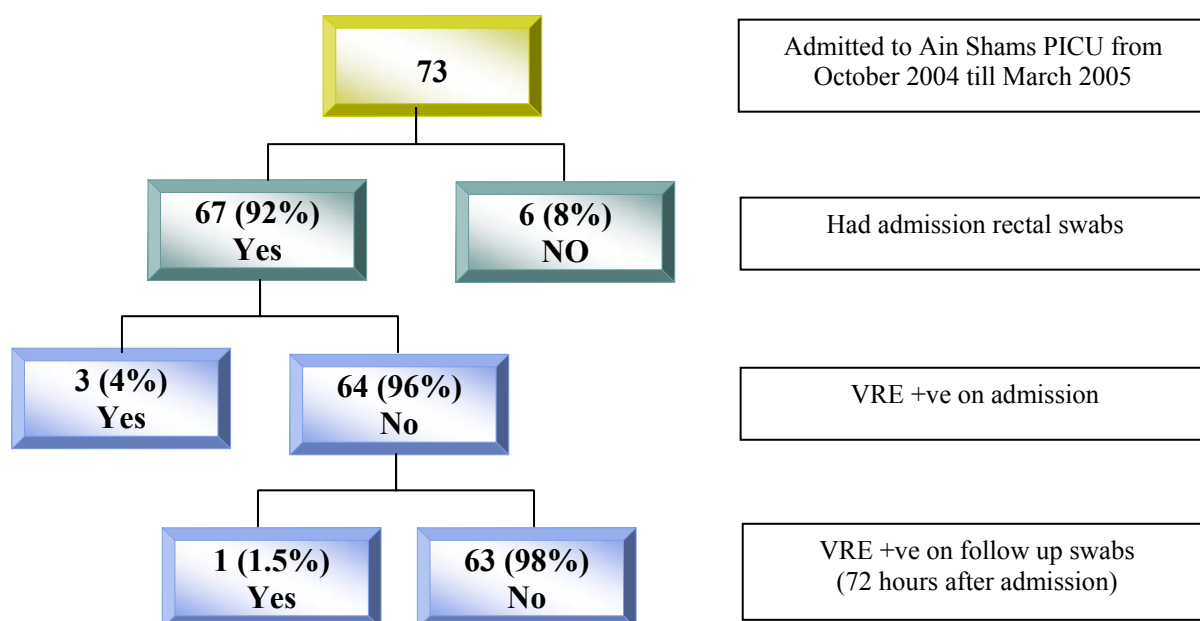
Risk factors associated with being colonized with MRSA on PICU admission are listed in table 1. Pre-PICU length of hospital stay of 3 or more days ( $p < 0.001$ ) (Relative risk (RR) = 3.8, 95% Confidence Interval (CI) = 1.7-5.9) and more than 2 admissions to the study hospital within the 12 months before the current admission ( $p < 0.001$ ) (RR= 3.2, 95%CI = 2.1-4.3) were associated with MRSA colonization at the time of PICU admission. As for VRE colonization, no statistically significant risk factors were detected.



**Figure 1:** Distribution of the study population as regards MRSA acquisition

<sup>1</sup> Three of them (18%) had associated respiratory tract infections due to MRSA and one (6%) had positive blood culture for MRSA and died.

<sup>2</sup> Five of them had symptomatic urinary tract infections due to MRSA



**Figure 2:** Distribution of the study population as regards VRE acquisition

**Table 1:** Comparison of patients admitted to the PICU (67 children) who were and were not colonized with MRSA on *ADMISSION*.

Variable	MRSA positive 17 Children No. (%)	MRSA negative 50 Children No. (%)	P value*	Relative Risk -RR- (CI 95%)
Age (years) Mean $\pm$ SD (range)	7 $\pm$ 3.5 (3-11)	6.5 $\pm$ 4.3 (0.5-13)	0.3	
Male	9 (53%)	27 (54%)	0.73	
Female	8 (47%)	23 (46%)	0.65	
Hospital admission in the previous 12 months None ( <b>Reference Category</b> )	7 (41%)	31 (62%)	0.02	
1-2 admissions	2 (12%)	13 (26%)	0.70	
> 2 admissions	8 (47%)	6 (12%)	<0.001	3.2 (2.1-4.3)
Pre PICU hospital stay $\geq$ 3 days	9 (53%)	13 (26%)	< 0.001	3.8 (1.7-5.9)
Mean PSI score	24	20	0.02	2.1 (1.9-5.0)
Pre PICU antimicrobial use				
Anti gram - ve	4 (23.5%)	12 (24%)	0.65	
Anti gram + ve	7 (41%)	18 (36%)	0.153	
Anti anaerobic	0 (0%)	1 (2%)	0.49	

Student's t-test for continuous variables and Pearson's Chi square test for categorical variables.

Of the 50 study patients who had an initial negative surveillance cultures for MRSA, 19 patients (38%) were subsequently colonized with MRSA during the PICU stay. While out of 64 study patients who had an initial negative surveillance cultures for VRE, only one patient (1.5%) was subsequently found to be VRE colonized during PICU stay [Figure 1&2].

Table 2 shows comparison of patients admitted to the PICU who did and did not acquire MRSA during their PICU stay. Risk factors for MRSA acquisition in the PICU were pre-PICU Gram positive antimicrobial use namely "cephalosporins", use of mechanical ventilator during ICU stay and age younger than 3 years. Another frightening observation was that 11 patients (57%) of the MRSA recently colonized patients during the PICU stay also were vancomycin-resistant [VRSA].

**Table 2:** Comparison of non colonized patients on admission to the PICU (50 children) who did and did not acquire MRSA during PICU STAY

Variable	MRSA positive 19 Children No. (%)	MRSA negative 31 Children No. (%)	P value*	Relative Risk -RR- (CI 95%)
Age (years) Mean±SD (range)	3±2.6 (0.5-6)	8±3.8 (3-13)	0.032	3.2 (2-4.4)
Hospital admission in the previous 12 months None ( <b>Reference Category</b> )	10 (52%)	21 (67%)	0.68	
1-2 admissions	6 (31%)	7 (23%)	0.82	
> 2 admissions	3 (17%)	3 (10%)	0.75	
Pre PICU hospital stay ≥ 3 days	5 (26%)	8 (26%)	0.65	
Use of Vascular Catheter(s)	19 (100%)	31 (100%)	0.98	
Mechanical Ventilation	9 (47%)	2 (6%)	<0.01	2.4 (1.5-3.8)
Pre PICU antimicrobial use				
Anti gram - ve	2 (10%)	10 (32%)	0.83	
Anti gram + ve	16 (84%)	2 (6%)	<0.001	4.1 (2.5-5.6)
Anti anaerobic	0 (0%)	0 (0%)	0.95	

\* Student's t-test for continuous variables and Pearson's Chi square test for categorical variables.

The predictive power of the model factors for MRSA colonization at the time of PICU admission is detailed in table 3. The presence of at least one of the risk factors for MRSA colonization at the time of admission had a sensitivity of 94% and a specificity of 46% and this was the case for 62% of the entire study population.

**Table 3:** Sensitivity and Specificity of a VARIABLE to predict MRSA colonization among admissions to the PICU

Variable	Sensitivity	Specificity	% of PICU admissions
<b>One Predictor</b>			
Variable Present			
<b>A- Pre PICU hospital stay <math>\geq</math> 3 days</b>	61%	76%	32%
<b>B- Hospital admission in the previous 12 months</b>	78%	63%	43%
<b>C-PSI</b>			
Critical illness score	80%	65%	52%
<b>Two Predictor</b>			
Variables Present			
<b>A&amp;B</b>	69%	66%	41%
<b>A&amp;C</b>	81%	59%	48%
<b>B&amp;C</b>	93%	48%	61%
<b>Any Predictor</b>			
Variable Present	94%	46%	62%

Five of the patients who acquired MRSA during their PICU stay developed infection. Four patients of them (21%) had positive urine cultures for MRSA, one (5%) had a positive sputum culture (table 4).

**Table 4:** Infections that developed in the children who acquired MRSA and/or VRE during PICU stay

Organism	No. of children colonized No. (%)	No. of children infected No. (%)	Site of Infection*
MRSA	19 (100%)	4 (21%)	Urinary Tract
		1 (5%)	Respiratory
VRE	1 (100%)	None	None

\* Infected children may have an infection at more than one site

## DISCUSSION

A high prevalence of MRSA colonization on admission (25%) has been discovered among patients admitted to the PICU in the present study. This high prevalence rate was significantly associated with being admitted to the hospital within 12 months prior to the current admission, having stayed in the hospital for 3 days or more prior to the PICU admission as well as a high PSI (table 1).

Furthermore, an extremely high rate of MRSA acquisition among PICU patients (38%) was also found. These findings are similar to those of **Korn et al., (2005)** where 24% of PICU patients were colonized with MRSA on admission and 42% of susceptible patients became colonized during PICU stay. However, the acquisition rate of the current study was slightly higher than that obtained by **Merrer et al., (2004)** who found an acquisition rate of 23% among PICU patients.

These findings suggest that a significant amount of nosocomial transmission of MRSA occur in our institution, both in and out of PICU. Another potential explanation for this observation may be that patients who are frequently admitted to acute care facilities are also commonly admitted to skilled nursing facilities, which are known reservoirs of MRSA (**Glenn et al., 2004 and Laoyan et al., 2005**).

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Acquisition of MRSA during PICU stay in the present study has been shown to be significantly associated with being younger than 3 years, use of mechanical ventilators and using anti Gram-positive antibiotics prior to PICU admission (table 2).

Previous individual exposure to antimicrobials particularly vancomycin (**Bonilla et al., 2001 and Bhorade et al., 2004**) and cephalosporins (**Girou et al., 2004 and Mulhausen et al., 2004**) has been associated with relatively high rate of colonization of MRSA (**Bonten et al., 2002**). Although **Fridkin et al., (2004)** suggested that the spread of MRSA is primarily due to patient transfer by health care workers (contaminated stethoscopes in 69% of cases and contaminated white coats in 54% of cases and contaminated hands in only 27% of cases) yet antibiotic use helps to maintain MRSA by providing a selective advantage.

On the other hand, **Fischer et al., (2005)** stated that antibiotic exposure might be a good predictor of MRSA incidence within a given population. In addition, other studies have shown that selective surveillance for MRSA among high risk patients reduces the transmission of that organism within an acute care setting and it is cost effective (**Gordts et al., 2003 and Girou et al., 2005**).

Being costly for our institution to perform active surveillance of all PICU admissions. An alternative method would be to screen only patients who had one or more significant risk factors for MRSA colonization and acquisition.

Moreover, in this study, a strategy to isolate and screen patients based on three readily available criteria [admission to our hospital within the past year, pre PICU hospital stay of 3 or more days prior the current admission and high PSI] would detect MRSA colonization with a sensitivity of 94% (table 3). However, the clinical effectiveness and cost effectiveness of this approach for control of MRSA colonization needs to be tested by further studies.

Because of limited resources, we could not include patients admitted for less than 48 hours in our study. These patients could have been an unrecognized reservoir for MRSA transmission to other patients. In addition, observation of the staff compliance with contact-isolation procedures as gloves and adequate hand washing after leaving an isolated patient room was difficult to perform.

A striking observation was that 57% of the MRSA strains isolated from the newly colonized patients were also vancomycin resistant (VRSA). This is in contrast to that shown by **El Kholly et al., (2003)** in a survey of five hospitals in Cairo, where none of the *Staphylococcus* strains isolated was vancomycin resistant. In another survey done on different departments of Ain Shams University Hospitals, although none of the isolated *staphylococci* was vancomycin resistant, yet 4.6% of all MRSA isolates were vancomycin intermediate *Staphylococcus aureus* (MIC $\geq$ 8ug/ml) (VISA) and 10.3% of isolates were heterogeneous VISA (**Sayed, 2005**).

High prevalence of MRSA and wide range of vancomycin use are both thought to be risk factors for VRSA (**Perl, 1999**). This makes the wide spread dissemination of these organisms an alarming realistic problem. With the emergence of *Staphylococcus aureus* with reduced susceptibility or resistance to vancomycin in our hospitals, strict adherence to current guidelines for vancomycin use and infection control practices is necessary to limit the impact of these multi-resistant organisms.

On the other hand, a very low rate of VRE prevalence among patients admitted to the PICU (4%) and even a lower rate of VRE acquisition (1.5%) were found in the present study. In another survey done on five hospitals in Cairo (**El Kholly et al., 2003**), 4% of all *Enterococcus* isolates were vancomycin resistant. These rates were much lower than that obtained by **Glenn et al., (2004)** who found that 18% of PICU patients were colonized with VRE on admission and 25% of susceptible patients became colonized after PICU admission. In addition, **Laoyan et al., (2005)** found that 23% of PICU patients were colonized with VRE on admission and 49% of susceptible patients became colonized during PICU stay.

In conclusion, MRSA acquisition occurred frequently in PICU, thus control of MRSA would be accomplished by screening for MRSA on admission to identify imported cases. Moreover, education concerning the importance of infection control measures as proper aseptic technique

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concerning mechanical ventilation and barrier protection from colonized patients as well as strict limitations for use of antimicrobials should be emphasized.

The present study also suggests that better understanding of the prevalence of colonization with resistant organisms especially among newly admitted PICU patients is a crucial step in infection control. Moreover, the identification of risk factors for colonization in this important age group population allows for development of novel and better strategies for control.

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خطورة الاستعمار بكتريا المكورات العنقودية المقاومة للميثيسيلين و المكورات المعوية المقاومة للفانكوميسين  
فى مرضى الرعاية المركزة للأطفال فى مستشفيات جامعة عين شمس

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