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Determining resistance status and intensity of dengue vector *Aedes aegypti* (l 1762) *(Diptera: culicidae*) to deltamethrin in the Gizan City, southwestern Saudi Arabia

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Abstract
In Saudi Arabia, the effectiveness of controlling dengue vectors is being greatly compromised by insecticide resistance (IR). This study investigates the status and levels of IR in *Aedes aegypti* (L., 1762) (*Diptera: culicidae*) in response to deltamethrin in Gizan City, Jazan region, southwestern (SW) Saudi Arabia. Larvae and pupae were collected using the dipping method during the winter season (Nov. 2021 to January 2022) from different breeding sites in the Gizan City, transferred to the insectary of the Saudi Center for Disease Prevention and Control (SCDC), and maintained under rearing conditions until the adult stage. Female mosquitoes, aged 2 to 5 days, were exposed to the World Health Organization (WHO) diagnostic dose (1×DD) of deltamethrin (0.05%) to assess baseline susceptibility. For populations exhibiting resistance at the diagnostic dose, an increased concentration equivalent to five times the diagnostic dose (5×DD; 0.25%) was applied to determine resistance intensity in accordance with WHO insecticide resistance (IR) intensity bioassay guidelines. The mortality rate for deltamethrin (0.05%) at the diagnostic dose 1×DD was 91%, which is below the 98% susceptibility threshold.
Assessment of resistance intensity indicated a low level of resistance in *Aedes aegypti* populations from Gizan City, with 98% mortality observed at 5×DD.
The present study indicates a low level of resistance to deltamethrin in the Gizan City. These results provide policymakers crucial information in designing and implementing effective insecticide resistance management (IRM) strategies. Further research is warranted to assess the efficacy and underlying mechanisms of resistance, particularly the role of the synergist piperonyl butoxide (PBO), in enhancing the control of pyrethroid-resistant dengue vectors.

Keywords: *Aedes aegypti*, Insecticide resistance, Resistance intensity, deltamethrin, Gizan City, Saudi Arabia

1. Introduction
Dengue Fever (DF) is a serious worldwide health concern. It is the most significant infection in humans [1]. There are four antigenically related, but immunologically distinct, serotypes of the dengue virus (DENV), which is a viral illness that belongs to the Flaviviridae family and Flavivirus genus, these are: DENV-1, DENV-2, DENV-3, and DENV-4 [2,3].
Currently, 50 million DF occur annually in around 100 countries across the Americas, South East Asia, Eastern Mediterranean, Western Pacific, and Africa, with 22,000 deaths primarily affecting children [4]. Dengue-related deaths vary yearly and by source, with figures such as 100 million infections resulting in 21,000 deaths [5] and recent estimates of 14.1 million cases and 9,508 deaths in 2024 [6]. Other sources indicate higher figures, with some modelling estimating 390 million infections leading to up to 36,000 deaths annually [1].
In 1994, Jeddah, Saudi Arabia, experienced a DF outbreak that resulted in 289 confirmed cases [7]. During the peak of cases in the summer and throughout the rainy season at the year's end, DENV-2 and DENV-1 were isolated. In 1997, DF re-emerged in Jeddah again with the identification of DENV-3 during the rainy season. The following year, in 2004, marked the first DF outbreak in Makkah, with DENV-2 and DENV-3 being isolated [8].
The subsequent outbreaks occurred in Jeddah in 2006, leading the Saudi Preventive

Department within the Ministry of Health (MOH) to launch a comprehensive strategy to control the disease [9]. In 2008, the first cases were reported in Al-Madinah, where DENV-1 and DENV-2 serotypes were detected [10]. By 2009, the Saudi MOH recorded 3,350 cases of dengue fever (DF) in the Kingdom, with an estimated case fatality rate of 4.6 per 1,000 [11]. The resurgence of DF in Saudi Arabia is linked to increasing urban development, along with intensified international trade and travel. Vector control (VC) programs worldwide are facing the issue of mosquitoes developing resistance to current insecticides. Adulticiding remains a critical strategy for preventing dengue; however, there is limited information on insecticide resistance among adult *Aedes aegypti* in the Middle East Region [12].

Space- spraying serves as a crucial emergency response tactic to help mitigate outbreaks of the DENV [13, 14]. VC strategies encompass the application of various types of insecticides to manage mosquito vectors that transmit viral and parasitic diseases. Different insecticides, varying in efficacy, have been utilized against multiple mosquito species. In particular, synthetic pyrethroids are widely employed in public health to manage disease vectors and agricultural pests because of their low toxicity to mammals and strong insecticidal properties [15]. Deltamethrin is an extremely effective pyrethroid insecticide that is utilized for controlling bothersome household mosquitoes, as well as disease-causing vectors [15]. In Saudi Arabia, deltamethrin ranks among the most commonly utilized insecticides in public health initiatives directed at controlling mosquito vectors [16].

The use of insecticides for vector control (VC) in Saudi Arabia dates back to 1948, when interventions were primarily directed at controlling Anopheles mosquitoes due to the high burden of malaria [17, 18].

Pyrethroids have been the most commonly applied insecticide group in Saudi Arabia due to their high effectiveness, fast knockdown, and minimal mammalian toxicity [13, 19]. But it has been reported in some regions, e.g., Makkah, Jeddah, and Jazan, where *Aedes aegypti*, the primary vector of dengue, has demonstrated significant resistance
to various compounds of this class [16, 20-22]. Pyrethroids are presently the only WHO-recommended insecticide class for indoor residual spraying and space spraying applications [23].

Widespread use of pyrethroids has increased the selection pressure on mosquito populations, leading to the emergence of resistance mechanisms such as knockdown resistance (kdr) mutations and metabolic detoxification pathways [24, 25].

The aforementioned resistance mechanisms have been documented extensively in *Ae. aegypti* infestations across Saudi Arabia, rendering conventional control strategies less efficient and dengue transmission risk greater [16, 21, 22, 25]. The rise of this resistance highlights the critical necessity for implementing integrated vector management (IVM) approaches and the introduction of new insecticides with new modes of action that can minimize the public health burden of vector-borne diseases.

As far as we know, no studies have been conducted/published in Saudi Arabia to quantitively evaluate the resistance density of *Ae. aegypti* to deltamethrin. Our current study aims to assess the susceptibility status and quantify the resistance intensity of *Ae. aegypti* collected from one of the endemic dengue foci in KSA, Jazan, to an important chemical, the pyrethroid deltamethrin.

2. Materials and methods

2.1 Study area

Gizan City is situated in the Jazan region, which spans approximately 22,000 km² and has a population of 1.6 million, located between 16◦54034.8588” N and 42◦3404.4472” E. Jazan lies in the subtropical zone in the southwestern part of Saudi Arabia. It is bordered by the Red Sea on the west, the Arab Republic of Yemen to the south and east, and the Asir region to the north. The region features a coastal length of 250 km along the Red Sea and shares a 120 km border with Yemen (Fig. 1). It contains over 3,000 villages scattered across the landscape, as well as around 100 islands in the Red Sea, including the Farasan Islands. The area's topography can be distinctly categorized into three sections:

(a) the Sarwat mountain range sector, which rises to the east (up to 2,500 m above sea level); (b) the hilly middle sector, which is 300-600 m above sea level; and (c) the coastal sector, which is located to the west (30 m above sea level). The weather is subtropical, characterized by an annual temperature of about 35 °C, with relative humidity ranging from 50 to 70%, and annual rainfall measuring 165 mm in the coastal region and 300-500 mm in the Sarwat mountain ranges [26, 27] GASTAT, 2017: https://www.stats.gov.sa/en/5655).

2.2. Mosquito collection

Ae. aegypti larvae and pupae were collected by the dipping method during the winter season (November 2021 to January 2022) from different breeding sites of the Gizan City, transported to the insectary of the Saudi Center for Disease Prevention and Control (SCDC), and maintained in rearing conditions up to the adult stage. The emerging adults were identified morphologically using available identification keys for KSA [28], and only the females were bioassayed.

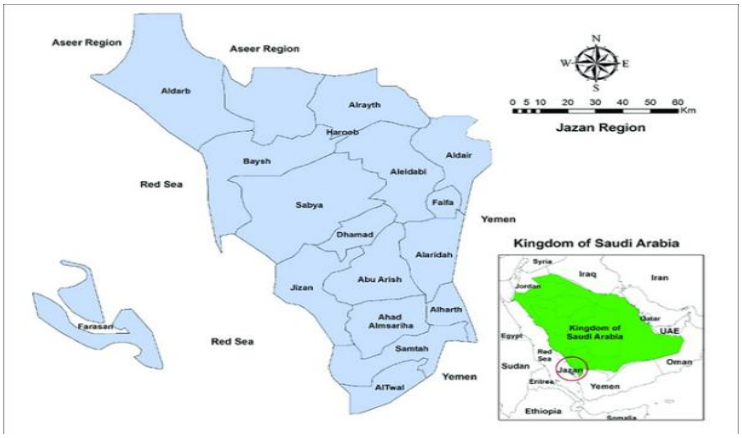

Fig 1: Map of the Jazan region, southwestern Saudi Arabia

2.3 Insecticide Bioassay

Susceptibility testing was conducted under optimal conditions (25-27°C and 70-80% relative humidity) following the standard protocol of WHO [23]. The WHO kits (insecticide-impregnated papers) were supplied by Universiti Sains Malaysia. Deltamethrin at a concentration of 0.05% was assessed at diagnostic doses (1xDD) as indicated by the WHO [23]. Additionally, a 5-fold diagnostic dose (5xDD) of deltamethrin (0.25%) was utilized to yield data on the intensity of resistance (IR) in those females that exhibited resistance at 1xDD. Four groups of 25 unfed female mosquitoes (5 days old) were exposed to the impregnated papers for a duration of 1 hour. The knockdown (kd) rate of the exposed mosquitoes was recorded after 1 hour of exposure (Knockdown time, KdT). Batches of of 50 females exposed to untreated papers served as controls. Mortalities were recorded after a period of 24 hours, and the susceptibility status of the mosquito populations was classified according to the WHO guidelines [23].

2.4 Data Analysis

2.4 Data Analysis The percentage of mortality was derived from the bioassay results. The WHO criteria were employed to differentiate between resistance and susceptibility status, as well as the IR of the mosquito populations tested [23]. If mortality exceeds 98% at 1xDD, the population is categorized as susceptible (S), and if it falls below 90%, it is classified as "resistant" (R). A mortality rate of 98-100% at the 5xDD indicates a low intensity of resistance (LRI).

3. Results

The mortality rate for deltamethrin (0.05%) at the diagnostic dose of 1×DD was 91% (Table 1), which falls below the WHO susceptibility threshold of 98%. The assessment of the resistance levels revealed a low resistance in Ae. aegypti populations from Gizan City, as indicated by a 98% mortality rate at 5xDD.

Table1. Bioassay of *Ae. aegypti* (Field and Laboratory strains) to deltamethrin 0.05% and 0.25%

Insecticide	Dose (%)	Field strain				Laboratory strain			
		N	%M	%Kd	St.	N	%M	%Kd	St.
Deltamethrin	0.05% (1X)	100	91	90	R	100	100	95	S
	0.25% (5X)	100	98	96	LR	-	-	-	-

N: Total numbers of mosquitoes; %M: Mortality percentage; %Kd: Knockdown percentage; St: Resistance status; S: Susceptible; R: Resistant; LR: Low resistance.

Discussion

The present study confirms that, *Ae. aegypti* in the Gizan City of Saudi Arabia have developed resistance to deltamethrin (0.05%) at the diagnostic dose (1×DD) with mortality (91%) below the WHO cut point of 98% [23]. When exposed to five times the diagnostic dose (5×DD), mortality increased to 98%, describing the resistance as low intensity according to WHO criteria [29].

These findings are in line with previous studies on pyrethroid resistance among *Ae. aegypti* populations in western and southwestern Saudi Arabia, including Makkah, Jeddah, and Jazan [30, 21, 16]. Aziz *et al.* (2011) [30] initially documented reduced mortality to deltamethrin (0.05%) in Makkah, while Al-Sheikh *et al.* (2016) [21] observed varying patterns of resistance in Jazan, where populations ranged from 80-95% mortality at the diagnostic dose. Al-Nazawi *et al.* [16] extended these findings further by coupling phenotypic assays with genotyping and demonstrating target-site knockdown resistance (kdr) mutations F1534C, S989P, and V1016G specifically to have a key function in pyrethroid resistance in Saudi *Ae. aegypti*. These mutations have subsequently been detected at moderate to high frequencies cross numerous locations, including Jazan, through molecular surveys [22, 31-33].

The results of the recent study on susceptibility to deltamethrin align with local data [34], which revealed that, *Ae. aegypti* is resistant to 0.05% deltamethrin in Jeddah City, western KSA. The result is also consistent with the findings of other authors in Thailand who reported the resistance of *Ae. aegypti* to deltamethrin [35].

On the other hand, The potential for resistance of *Ae. aegypti* to deltamethrin contradicts findings by researchers in India, who determined that adult *Ae. aegypti* and *Ae. albopictus* were susceptible to deltamethrin [36].

This study showed a low intensity resistance of *Ae. aegypti* to the diagnostic dose (5× DD), suggesting low resistance as defined by WHO [23]. This result is somewhat consistent with the findings reported by Al-Koleeby *et al.* (2020) [37] in Yemen, where they observed low mortality rates in a pyrethroid-resistant population of *An. arabiensis* exposed to deltamethrin at 5 times the diagnostic dose (5xDD. In Guinea-Bissau, the phenotypic bioassays revealed deltamethrin resistance in the *Anopheles* mosquito population according to WHO guidelines (WHO, 2022), and intensity testing indicated that resistance is of moderate to high intensity [38].

The resistance of *Ae. Aegypti* to deltamethrin could have resulted from cross-resistance with DDT which was used previously in VC programs. Moreover, household aerosol insecticides, where the main active ingredients are pyrethroids, could have contributed to the species' resistance [39].

The low intensity of resistance discovered in this research has operational implications. Per WHO guidelines [29], low-intensity resistance could not drastically undermine vector control effectiveness when practically applied concentrations of insecticides are adequate. However, continued reliance on pyrethroids, particularly under space spraying and IRS, might continue to select for resistance [19, 24]. Integrated resistance management encompassing rotation of insecticide classes, use of larvicides with different modes of action, and non-chemical control should therefore be accorded highest priority to prevent upgrading from low to moderate or high levels of resistance.

Moreover, the dramatic knockdown (Kd) response at both intensity and diagnostic doses (90-96%) suggests that while resistance does occur, physiological processes (e.g., metabolic detoxification by cytochrome P450 monooxygenases) could be involved in concert with kdr mutations [16, 40]. Future research in Jazan should thus include synergist bioassays (e.g., pre-exposure to piperonyl butoxide), dose-response bioassays (LC50, LC90), and molecular diagnostics to better

define the landscape of resistance mechanisms.

Overall, this research provides the first quantitative assessment of deltamethrin resistance intensity in *Ae. aegypti* in Gizan, as low intensity but suggestive of ongoing selection pressure. Continuous surveillance, mechanism explanation, and pre-emptive resistance management are essential in ensuring the efficacy of pyrethroid-based dengue vector control in Saudi Arabia.

The World Health Organization (WHO has put forward several recommendations to assist countries in developing and executing plans for tackling insecticide resistance [41]. When resistance to a specific insecticide is identified, VC initiatives can primarily resort to other classes of insecticides to alleviate selection pressure and hinder the dissemination of resistance. There are four primary categories of insecticides utilized in public health control initiatives: pyrethroids, organochlorines (mostly DDT), organophosphates (OPs), and carbamates. It is noteworthy that pyrethroids and DDT operate through similar mechanisms, which makes OPs and carbamates vital for successful resistance management strategies. Several tactics, such as alternating insecticides, employing mosaic patterns, and pairing pyrethroids with OPs or carbamates for indoor residual spraying (IRS), have shown remarkable results [13, 42, 43]. Therefore, it is critical to comprehend the mechanisms behind resistance.

The outcomes of the present study can be considered by VC programs for

preventative dengue control in the region, as a component of temporal rotations or spatial mosaics to manage deltamethrin and other insecticide resistance.

The concerningly low resistance of *Ae. aegypti* to deltamethrin raises significant issues for the effectiveness of control strategies that rely on pyrethroids.

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